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Making disaster relief more effective

EACH year tens of thousands of people die as the result of large scale disasters such as earthquakes, famines, floods and so on. Some years disaster reaches dreadful proportions; no-one will ever know how many were killed in the Tangshan earthquake of 1976, but rumours of 700,000 have never been denied. And while the number of major disasters changes little from year to year, there is some evidence that the proportion of the population which die in these disasters has risen in recent years, even though research into the prediction, prevention and mitigation of disasters has gone on apace for many years and has had some substantial successes. Many deaths and much of the misery that attends on disasters is unavoidable in the foreseeable future, but very often a good relief and supportive operation after the disaster has struck could make a major contribution both to saving lives and alleviating distress. But is relief well enough done? The International Disasters Institute, just established in London, suspects it is not and hopes to stimulate research into how it can be done better.

The institute has emerged in an interesting way as the formalisation of links between groups of predominantly young research workers with interests ranging across many specialisations from architecture to nutrition. Until recently they had attachments, of varying degrees, to the London Technical Group, Bradford University's Disaster Research Unit and the Oxford Polytechnic's Disasters and Human Settlements Unit; the institute replaces these as a focus for their work. London is widely regarded as an excellent place for such an international institute to be established because so much of the expertise and information sources of an old colonial power is still available.

To a certain extent the institute is bound to find itself in conflict with the relief-giving agencies and governments' own disaster organisations; not because the institute will be in the business of relief itself, but because one of its most important functions, according to Dr Frances D'Souza, its director, will be in the evaluation of the effectiveness of their operations. Recent years have, of course, seen some spectacular and often-quoted blunders in relief, and it may be that public knowledge

of these has made it perceptibly more difficult for the agencies to raise money. But there have been other less spectacular ways in which apparently sensible and desirable relief operations have (as John Rivers reported in *Nature*, January 12, page 100) been aimed at the wrong targets. Massive inoculation campaigns and the shipping in of disaster shelters are two such activities which have come in for much criticism. It is undeniable that relief operations save many lives; the question the institute asks is whether a long cool look at them might not increase their effectiveness.

One of the prerequisites for doing this is that those involved in relief should neither see disasters as a means of changing the social system nor should they perpetrate the white-man-with-megaphone image. Relief workers should recognise that disasters as such are not a product of the modern age, even if the scale of fatalities might be unprecedented; similar occurrences must always have been a feature of life in some parts of the world. There is thus a lot of handed-down expertise and toughness to weather the aftermath of a disaster. Relief can only be truly effective if it is supportive of this local expertise and enterprise, not disruptive of it.

Will the institute flourish? It will frequently find itself unpopular, but popularity is, presumably, hardly a thing that those associated with it have actively courted for the past several years. The most formidable obstacle at present is money. The institute operates out of a London basement, where it keeps a library of books and reports. It employs a handful of staff and it needs £30,000 per year to keep going; this is core funding and does not cover the costs of projects the institute may try to stimulate, for which separate money will be sought. The problem is that raising money for the immediate needs of disaster-struck people has a definite attraction to it, whereas raising money to pay for the administration of an organisation that tries to evaluate such operations lacks a certain public appeal. All the more reason why companies, foundations and individual people (notably scientists) who recognise the unspectacular but desirable role of painstaking research should regard the International Disasters Institute as particularly worthy of their support. □



Supreme Court nullifies patent ruling on living organisms

David Dickson reports on how a decision about the patentability of computer programs has implications for recombinant DNA technology

In a one-line decision that could have important implications for the future development of industrial processes using biological techniques, the US Supreme Court last week ordered an appeals court to reconsider its decision that living organisms can be patented.

Although the exact meaning of the decision is now being hotly debated in Washington, many feel that it is a signal from the Supreme Court that since there is no explicit mention in existing patent legislation that living organisms are covered, the issue of their patentability should be resolved in Congress, rather than by the courts.

In the short term, the court's action means that a number of patent applications for aspects of recombinant DNA techniques, the decisions on which have been held up pending a definitive outcome of the case in question, are likely to remain unresolved for a number of months to come.

The Supreme Court's decision was made on a dispute over a patent which had been applied for by a group of scientists working with the pharmaceutical manufacturer Upjohn Co. for a naturally occurring microorganism, *Streptomyces vellosus*, which the scientists had isolated, purified and were

using to produce the antibiotic lincomycin.

The Patent and Trademark Office had initially turned the patent application down, primarily on the grounds that the patent laws as they now stand contain no indication that they are meant to cover living organisms; in support of this claim, it was argued that if Congress had meant such organisms to be covered, it would not have been necessary to pass a separate Plant Patent Act, as was done in 1930.

Last October, however, in a widely publicised verdict, the US Court of Customs and Patent Appeals reversed the decisions and allowed the patent. The court argued that despite the fact that microorganisms were alive, since they had become an important tool in chemical and pharmaceutical industries, "we do not see any reason to deprive it, or its creator or owner, of the protection and advantages of the patent system".

This ruling was subsequently used as the basis of a further decision by the appeals court in March of this year, which upheld a patent application made by an Indian microbiologist working for General Electric, Dr Ananda M. Chakrabarty, for a *Pseudomonas* bacterium which had been genetically altered to produce enzymes capable of degrading oil—and hence might provide a useful way of combating oil spills.

If the Supreme Court had upheld the ruling in the Upjohn case—the Justice Department has yet to decide whether to consult it in the second case, although this now seems increasingly likely—it would have set a precedent for granting a wide range of patent applications in the general field of biotechnology, including genetic engineering; and as one patent attorney said in Washington last week "the case would have been closed".

As it is, the court did not take a stand on the issue, but has virtually returned the debate to square one by referring it back to the appeals court, asking that it be re-examined "in the light of" a judgement handed down the previous week in a case over the patentability of computer programs.

In this case, a computer engineer had applied for a patent on a method for updating fire alarms in a chemical engineering process, the only novel aspect of which was the inclusion of a mathematical algorithm (which previous cases had established was not patentable on its own).

The application had initially been rejected by the patents officer on the ground that including an unpatentable step as a novel feature in an otherwise conventional manufacturing process did not make the process patentable. The CCPA disagreed, and reversed the

Lederberg suggests national foundation to exploit fruits of university research

A National Research and Development Foundation, which would own and exploit the patent rights to inventions made in universities with federal grants money, but at an arm's length from the universities themselves, has been suggested by Professor Joshua Lederberg, president of Rockefeller University in New York. The suggestion has been made in a letter from Professor Lederberg, Nobel Prize-winner and until last month professor of genetics at Stanford University's School of Medicine, in a letter to Senator Gaylord Nelson, whose select committee on small business is currently holding hearings into federal patent policy.

In his letter, Professor Lederberg says that he does not believe the pursuit of proprietary gains to be the proper business of the staff of a university.

"The possibility of profit—especially when other funding is so tight—will be a distorting influence on open communication and on the pursuit of basic scholarship" he said.

Professor Lederberg continues: "On the other hand, the need to protect development investment for the exploitation of inventions is absolutely sound, and essential to the nation's economy. Such investments are typically much larger than the costs of the original research, and are comparable to the expected 'profits'—when there is a pay-off.

"It should not be so difficult to reconcile these objectives, using the Research Corporation as a model. Set up an accountable, not-for-profit national R&D foundation, and vest all government-owned patents in it.

"NRDF will then enter the market, at arm's length, with licences etc. for the inventions it owns. The fees should first of all cover its operating expenses. Then it can use its profits and accumulated reserves to fund grants and contracts that will continue to further the practical applications of scientific discovery."

Professor Lederberg says that universities should not share in the licence fees except to the extent of their cost-sharing in the research that led to the invention, which would be assumed to be 10 per cent for routine cases.

Furthermore individuals should not, in principle, be rewarded for the results of work for which they were already receiving an academic salary, although neither should they be hindered in private arrangements, for the fruits of time and energy for which they were not on salary, and which are outside their normal academic duties.

"The financial and regulatory stresses on our private institutions are threatening their future existence. But patent-seeking is an inappropriate answer to these financial dilemmas," Professor Lederberg says.

David Dickson

decision; but two weeks ago the Supreme Court, in a six to three ruling, overruled the Appeal Court and agreed that a patent could not be awarded.

Patent attorneys are now discussing what the Supreme Court meant when it said that the Upjohn scientist's application should be re-assessed "in the light of" this verdict. One interpretation is that, using a direct analogy, a biological process cannot be patented if its only novel component (namely a living organism) is unpatentable.

However, some feel that the significant point lies in the Supreme Court's statement that its decision did not necessarily imply that computer programs (and by extension microorganisms) are unpatentable, but that existing patent law—which does not consider either—should be read literally. Decisions which imply extending its applicability should be taken by Congress, not the Courts.

How the Court's decision is likely to affect other patent applications, particularly in the field of genetic engineering, is at present uncertain. Of

those which have already been filed for recombinant DNA inventions made with the help of federal funds, the most directly related is an application from Dr Roy Curtiss, of the University of Alabama. This application covers not only the techniques for modifying *E. coli* to make it suitable for carrying out high risk experiments, but also includes the modified organism itself.

Less likely to be directly affected—apart from the delay caused while the legal arguments are sorted out—are applications for basic recombinant DNA techniques, such as a joint application pending from Dr Stanley Cohen, of Stanford University, and Dr Herbert Boyer, of the University of California, and two further similar applications from the University of California.

Whether or not the Appeals Court, having reconsidered the case, will stick to its original decision, it seems almost inevitable that the issue will return to the Supreme Court. The logic of last week's decision is that the ball will then be passed on to Congress (which is already preparing itself for such an

eventuality, and discussing under whose jurisdiction the issue should fall).

Dr Gilbert Omenn, Assistant Director of the Office of Science and Technology Policy, said this week that the administration would welcome guidance from Congress on how patent applications for living organisms should be handled by the courts.

As far as the industrial uses of genetic engineering are concerned, the outcome may be more of philosophical than economic interest. Most scientists argue that it is the techniques, rather than the microorganisms themselves, for which patent protection would be most valuable (although some feel that plasmids should be patentable).

However, in other areas of the industrial use of biological processes—such as the propagation of complete organisms from a single cell—the issues are more complex. Thus, as the political debate over safety guidelines for recombinant DNA experiments loses its momentum, a related debate on which opinions are just as diverse may soon take its place. □

Congress approves solar power satellites—and asteroid mining

DESPITE opposition from both the administration and environmentalist groups, the US House of Representatives has passed a bill for a major research and development programme that could lead to the design and construction of an array of vast satellites capable of collecting solar power and transmitting it to earth via a microwave link.

The House has agreed by a vote of 267 to 96 to a bill, actively supported by aerospace and utility companies, authorising the expenditure of \$25 million in the fiscal year 1979 for initial research and development on the solar satellite concept. Such a project could eventually involve putting more than a hundred solar collectors in orbit around the earth, and might cost over \$1,000 billion.

The administration has already proposed a much more modest \$15.6 million research programme into the feasibility of solar power satellites, spread over a three-year period ending in September 1980. It claims, however, that at present neither the economic necessity nor the technological feasibility of the project has yet been demonstrated sufficiently to warrant the type of public investment that Congress is suggesting.

Environmentalists are also concerned at the environmental hazards associated with the use of microwaves to beam the power from the satellites

down to the earth's surface. Many feel that the aerospace and power industries are merely pursuing their own self-interest; Representative Richard Ottinger (Dem-N.Y.) for example, the only member of the House Science and Technology committee to oppose the bill, has called the project "one of the greatest boondoggles of all time".

Under the terms of the bill, the Department of Energy and the National Aeronautics and Space Administration would be required to come up with plans for an expanded and accelerated programme for research and development into solar power satellites by next January. The agencies would also be required to study various environmental questions, including the effects of the microwave beams on people and on radio reception.

In a related decision, the Senate last week passed an authorisation bill for the National Science Foundation which included the provision that \$500,000 of the NSF's funds in 1979 should be spent on examining the feasibility of constructing solar power satellites.

Of particular concern to the administration, however, is the Senate's declaration that the feasibility study should consider orbiting structures "manufactured from lunar and asteroidal materials". This is a concept developed and popularised by Professor Gerald O'Neill of Princeton

University, but which some Congressmen feel pre-empts a whole range of questions about the future of the US civilian space effort.

David Dickson

Carter can kill fast breeder project

PRESIDENT Carter has won his first victory—albeit slim—in his efforts to get Congress to accept the termination of the liquid metal fast breeder reactor project at Clinch River in Tennessee. This is a project to which he has been consistently opposed, initially on non-proliferation grounds, more recently on technical and economic arguments.

The Senate Energy Committee, although agreeing to include \$159 million for the project in its Department of Energy authorisation for 1979, has agreed that the project can be terminated if the President feels this to be in the national interest.

However, if Clinch River is terminated, the committee has directed that the money be used for an alternative fast breeder design project for which Congress could later decide whether to proceed to production. Carter has in the past been adamant that he will not commit himself to anything more than a conceptual design study; so a presidential veto remains a possibility. □

R & D figures show US innovation study may be redundant

NEW estimates of the amount of money spent by US industry on research and development indicate that a major interagency study of industrial innovation announced by President Carter only two months ago may already be partly redundant.

The interagency study, which is being carried out by the Department of Commerce, was established largely at the instigation of Dr Frank Press, the President's science adviser. And a major source of concern brought to the administration's attention by Dr Press was the apparent decline in industrial investment in research and development, accompanied by a shift in emphasis from long-term to short-term goals.

However a survey of 624 companies, which between them account for 90 per cent of all privately-funded R & D performed by US companies, published last week in *Business Week* showed that the companies spent a total of more than \$18 billion on R & D in 1977, an increase of 16.4 per cent over 1976.

Even after allowing for inflation, the figures indicate a net increase in private R & D of 10 per cent. And in some industries, such as semiconductors, instruments, office equipment and "miscellaneous manufacturing", the figure was significantly higher.

Further evidence that the situation may not be as gloomy as some portray

is provided by a forecast survey made recently by McGraw Hill, which predicts that between 1977 and 1978 the proportion of R & D funds devoted to new products—a category of particular concern to the administration—will in-

crease from 26 to 33 per cent, while that devoted to the improvement of existing products, which had increased steadily from 48 per cent in 1975 to 59 per cent in 1977, will fall back again to 48 per cent in 1978. □

France lifts objections to renegotiating US-Euratom agreement

THE embargo placed by the US on licensing further exports of nuclear fuels and materials to Europe—including enriched uranium to research reactors in France and Germany—is likely to be lifted in the near future. This follows the French government's agreement to lift the objections to renegotiating the agreement between the US and Euratom which covers such exports.

The US had imposed the embargo from the middle of April, 30 days after President Jimmy Carter had signed the nuclear non-proliferation act. This act requires the re-negotiation of all bilateral agreements covering the export of nuclear fuels in the light of new safeguard provisions which determine whether an export licence will be granted.

The European Economic Commission in Brussels had originally drafted a letter to Washington agreeing to re-negotiate, but insisting that this agreement should not prejudice the

outcome of the negotiations. The draft also insisted that any new agreement should not pre-empt the outcome of the International Nuclear Fuel Cycle Evaluation (INFCE) study being undertaken by the International Atomic Energy Agency in Paris, the results of which are expected next year.

However the council of ministers had refused to let the letter be sent, largely at the instigation of the French government, who insisted that the US had no right to break the existing agreement unilaterally.

Following discussions between President Carter, President Giscard d'Estaing of France and Chancellor Helmut Schmidt of West Germany in Washington last month, the French objection has been withdrawn. And early this week Dr Guido Brunner, EEC Commissioner for Energy and Research, was drafting a new letter which, it is hoped, will allow re-negotiation to begin—and the export of nuclear fuels to be resumed. □

Delegation of top US science administrators visit China

IN a move to develop scientific and technological links between the US and China, the Carter administration has sent a top level delegation of science administrators to Peking for four days of talks.

The delegation, which was due to leave Washington today (6 July), is being led by Dr Frank Press, director of the Office of Science and Technology Policy. It also includes the heads of all the major civilian research funding bodies, including the National Science Foundation, the National Institutes of Health, the National Aeronautics and Space Administration, and the Department of Energy's Office of Energy Research.

Although there has already been much unofficial contact between scientists in the two countries—Dr Press himself headed a delegation to discuss co-operation in earthquake research in 1974, and a group of Chinese nuclear and plasma physicists is currently on a four-week visit to US universities hosted by the National Academy of Sciences—this is the first time that

China has agreed to official bilateral talks since contacts were re-opened in 1971.

The Peking talks will have four basic purposes:

- to establish official contacts in science and technology;
- to explain United States science policy;
- to assess the present state of science

and technology in China; and

- to suggest ways of expanding exchanges.

The White House has denied that the visit is in any way a signal to the Soviet Union—increasingly isolated by scientists in protest at the treatment of dissidents—or that it is an attempt to play the Soviets and the Chinese off against each other. □

Soviet science schools to go

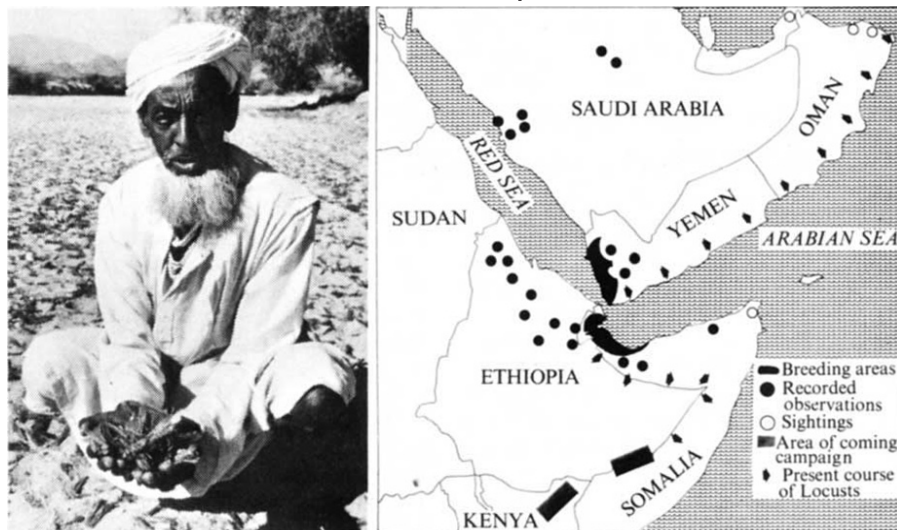
SPECIALISED schools, concentrating on mathematics, physics, chemistry and biology, which have for some considerable time been a feature of the Soviet educational system are to be phased out. The schools were intended to provide early training for the scientific cadres needed by the "scientific-technological revolution"; according to a recent report, however, many pupils graduating from these schools do not go into science at all but choose other occupations. The specialised schools for foreign languages (French, English,

German, Spanish and Oriental languages) will remain; presumably the majority of their pupils still proceed to become linguists and interpreters.

According to Moscow radio, school children will now be switched to an "abridged syllabus" in the natural sciences. However, at a recent meeting of the Academy of Pedagogical Sciences, its president, Academician, Professor V. N. Stoletov, affirmed that this change would not be reflected in the general educational standard of school-leavers. □

Locusts on the march again

Locust swarms are spreading in the Horn of Africa and the Arabian peninsula. **Chris Sherwell** reports.



A locust scout in Ethiopia (left) counting the number of dead locusts in a given area after aerial spraying. Sixteen years after the last major locust plague, surveying has become less rigorous and so less effective. Now that locusts are breeding rapidly and spreading some local organisations are not fully prepared.

DESPITE hopes only a month or two ago that the locusts were receding, the world's first serious desert locust plague in 15 years is now a real threat. Swarms have been building up in the Horn of Africa and the Arabian peninsula. The insects have managed to evade hamstrung efforts to confine them to the area and are now spreading south and east, endangering the food and livelihood of millions of people. New plans are being laid to combat the threat but millions of dollars' worth of assistance is needed.

Only last week a panel of locust experts advising the UN Food and Agriculture Organisation warned of the need for emergency action over the next three months, saying \$3 million-worth of assistance "must be provided immediately". Scientists at London's Centre for Overseas Pest Research (COPR) are closely involved in monitoring the locusts' progress and believe present conditions are similar to those prevailing immediately before the outbreak of the last serious plague in 1949. That lasted until 1963.

Good rains in the Sahel and at the eastern end of the Arabian peninsula in the middle of last year seem to be the underlying cause of the problem. The rains boosted the number of locusts which appeared later in October and November in the Red Sea and Gulf of Aden area. Further widespread and prolonged rainfall encouraged more breeding (the locusts' eggs need to take up free water in the soil in order to develop) and it soon became apparent that, without control measures, an un-

usually large number of locusts would be threatening the whole area this year.

Though both the Sudan and Saudi Arabia managed to take some steps, control efforts were hindered elsewhere by military engagements. War between Ethiopia and Somalia impeded ground control teams and severely limited aerial spraying operations in important locations during the early part of this year—a crucial period. Persistent outbreaks of hostilities since then (most recently in the past week), and instability in both North and South Yemen, have not lessened the difficulties. The long-running Eritrean conflict has complicated the problem further.

Reports from the area received in London last week indicate that one swarm in north-western Somalia covers some 400 to 500 sq km, another covers 360 sq km, and that together with about a dozen other swarms the total area infested by locusts in Somalia is about 1,600 sq km. Reports from Ethiopia have put the number of swarms there at 50, some of them well over 100 sq km, and though some spraying is being done the effort is thought to be too little, too late.

The damage the locusts are doing is enormous. This most-studied of insects—its activity has been documented for over 4,000 years—needs to eat its own weight each day (2 to 3 grams) of fresh food. A swarm covering 1 sq km might contain 40 million to 80 million locusts. This means hundreds of thousands of tonnes of vegetation are being devoured each day.

Further south in Somalia, and in

Kenya and Uganda, important cash crops such as bananas, tea and coffee could be affected, as well as staples like maize. Locusts' flying activity is affected by temperature, and under the cool conditions of the East African Highlands where such crops are prolific, the swarms would settle for days, causing particularly expensive damage.

Though the techniques of surveying have improved, an additional problem this year has been that, even allowing for difficulties of access, surveying and monitoring by local organisations has been less effective than in the past. This is partly because the period of "recession" over the past 15 years—the only interruption being a short-lived "plague" in 1968—has allowed local organisations to wind down.

Most of the responsibility for locust control over this period has thus passed upwards onto the shoulders of the Desert Locust Control Organisation of Eastern Africa (DLCOE), an international grouping of seven countries—Ethiopia, Somalia, Kenya, Tanzania, Djibouti, Uganda and Sudan. This has its headquarters in Addis Ababa and an aircraft base in Nairobi, but it has only seven aircraft, two of which are Norman-Britten Islanders from Britain which are still not ready to spray locust swarms. The rest are old Beavers. The DLCOE is acquiring two more Islanders and specially-equipped Land Rovers, all from Britain, but the FAO panel said in its statement last week that additional assistance was needed in the form of insecticide, camping equipment, light and heavy vehicles, sprayers and light spraying planes.

How far this can help before the annual locust cycle starts repeating itself is unclear, but urgent action is certainly needed. Swarms have been sighted as far east as Gujarat state in India, in Pakistan, in the Arabian Sea and all along the Arabian peninsula coast. Fears are also growing of a westerly movement into the Sudan, Chad and the Central African Empire, perhaps threatening countries as far west as Morocco.

The DLCOE, with help from the COPR and the FAO, is focusing its efforts on the locust swarms now breaking out of the northern part of the Horn around Djibouti and heading south carried on the prevailing winds. It is this development over the past fortnight which has caused the worries to mount, for the locusts will continue moving until they find more rain where they can breed. The experts are planning their next campaign for the Ogaden in October, provided hostilities do not break out there as well. If this fails, another campaign is already being prepared for northern Kenya. In 1954, the locusts moved as far south as Tanzania. □

Nuclear waste problem solved, claims Sweden's nuclear industry

"WE are not going to leave any waste around that is going to harm anybody." So says Asea-Atom's Dr Kåre Hanners, one of the authors of a just-published report which asserts that the Swedish nuclear power industry has found a way of safely storing unprocessed, spent fuel from nuclear reactors for hundreds of thousands of years.

According to the report, "Nuclear Fuel Safety Project 2", spent, unprocessed fuel would first be stored in water pools for 40 years. It would then be loaded into 4.5 metre-long canisters, each made of 15 tons of copper, with walls 20 cm thick. The space between the fuel and the canister walls would then be filled with lead, and each canister finally deposited in its own tunnel off a shaft drilled 500 metres down into bedrock.

After being drilled, each tunnel would have been lined with compressed bentonite, which swells to ten times its own volume on contact with water if it is not constricted. If it is constricted, as it would be in the tunnel, its pressure increases until it becomes almost totally impermeable. When the canister had been deposited in the tunnel, all the space left would be sealed with more bentonite, and a mixture of bentonite and quartz sand would be used to fill the shaft after all the tunnels were full.

In this way, the report maintains, corrosion of the canisters would be so small that it would not damage their integrity for thousands of years, and any waste which eventually did escape would be prevented by the action of the bentonite from being carried by the geological transport of water through the rock to the biosphere.

With the publication of this report, the nuclear power industry is making a final plea for its survival. Faced in 1977 by the new coalition government's

so-called Stipulation Law, requiring owners of nuclear reactors to show how and where they could store with complete safety either high-level reprocessed waste or unprocessed, spent fuel, the power industry decided to give top priority to showing that it could meet the challenge. All normal research and development within the industry stopped, while 200 to 300 of its own researchers as well as consultants from various universities concentrated all their energies on this one project.

The effort, which cost well over 10 million, has resulted in two reports: one, published last year, on the handling of spent fuel and final storage of vitrified, high-level waste, and this second one. The first report has been reviewed by many Swedish and foreign bodies and has mainly been approved, although there have also been many criticisms. These have often concerned the reprocessing agreement concluded on behalf of the industry with the French firm Cogema. The agreement is secret and not even the government's official reviewers of the safety of the industry's proposals have been allowed to see it: a plainly ludicrous situation. It is hardly surprising that French inexperience and inefficiency reportedly bordering on chaos in reprocessing the light water reactor fuel that Sweden uses, plus reports that the agreement contains an absolute escape clause for Cogema with no guarantee to reprocess at all in the event of major technical hindrances, and the fact that the agreement covers only reprocessing in the 1980s rather than for the whole 30-year lifetime of a new reactor, have raised the question of whether the contract can be "adequate", as demanded by the Stipulation Law.

Anti-nuclear groups have challenged the first report on other grounds as

well. They say, for example, that the canisters designed for transporting spent fuel to and from the reprocessing plant would not be able to withstand pressures of long immersion in case of shipwreck; that the storage facilities would need security measures that would infringe democratic rights; that various technical difficulties have been underestimated such as the sealing off of the openings to the final repository shafts to prevent leakage of ground water; that the stability of Swedish bedrock is not as great as the industry supposes; and that research findings unpalatable to the industry have been suppressed.

In the face of these assertions and counter-assertions between pro- and anti-nuclear groups, each of whom suspects by now even the motives and mentality of the other, and reflecting the extremely tight political situation in which the Prime Minister's party has sworn it will do away with nuclear power but faces enormous pressure in the opposite direction from its coalition partners, the government has still not pronounced on the overdue question of whether the loading of the Ringhals 3 and Forsmark 1 reactors can go ahead. Their owners have, under the Stipulation Law, applied for permission to load and to reprocess the spent fuel, and both reactors are standing ready for operation. Although Energy Minister Olof Johansson has promised to let them know in August and to present a new energy policy in December, it seems likely that the owners may be kept waiting even longer than that. In fact, on practically every aspect of current energy policy, the government could well adopt as its slogan the words of a former Australian Prime Minister: "The government's position is clear. It has not made up its mind." **Wendy Barnaby**

Shcharanskii trial delayed again, others exiled

ACCORDING to Soviet law, Anatolii Shcharanskii, the Moscow mathematician and human rights campaigner, who has been in custody facing possible treason charges since March 1977, should have been brought to trial not later than 15 December 1977. By special permission of the Supreme Soviet, this period of pre-trial intention was extended to 15 June 1978. Although this extension period has now expired, there are still no indications that Shcharanskii will either be brought to trial or released from custody in the near future.

Meanwhile, however, several of his fellow activists have been removed

from Moscow. On 17 May, Josif Begun, a cyberneticist, just returned from a sentence of two years' Siberian exile, was arrested on a charge of residing in Moscow without the necessary permissions. He has since been sentenced to a further 3 years exile.

On 1 June, Vladimir Slepak, an electronics engineer, and his wife were arrested for "malicious hooliganism" (in fact, for making a peaceful demonstration—a right specifically guaranteed under Article 50 of the new Soviet constitution). Slepak has now been sentenced to 5 years' Siberian exile, and it is feared that a similar sentence may be imposed on his

wife, who is at present in hospital with duodenal ulcers. Begun and Slepak are both to appeal against the sentences.

Like Shcharanskii, Slepak combined his involvement in the Jewish emigration movement with membership of the illicit "Helsinki Monitoring Group". His son told *Nature* recently that his father did not see how the Jewish demand for emigration could be divided from the overall human rights movement. "The right to emigrate is one of the fundamental human rights," he said. "So it is no good saying 'You eat your apple and I'll eat my apple.' Now we are both biting into the same apple!" **Vera Rich**

International research could ease world food problem

FUTURE research carried out by the Consultative Group on International Agricultural Research (CGIAR) could help lower the probability of extreme food shortages and stabilise international food markets. This could be achieved if food importing countries, whether developing or industrialised, applied new research results to increase their own food output and lessen their reliance on world food markets; and if exporters did the same to increase the food available to importers.

These are the conclusions of a report published last week called 'International agricultural research: potential impact on world food markets and UK agricultural strategy'. It was produced for the Centre for Agricultural Strategy at Reading University by A F McCalla of the University of California, who is researching into the effects of the CGIAR research programme on world food supplies.

Before drawing his conclusions, Dr McCalla is careful to point out that research is only one factor influencing world food output and that the CGIAR programme is only a small aspect of that research. In 1974, he says, an estimated \$3.8 billion was spent on agricultural research throughout the world. The developing countries accounted for 15% or \$0.57 billion of this expenditure, of which \$50 million or 10% was spent by the CGIAR. The CGIAR was therefore responsible for no more than 2% of the total world expenditure on agricultural research.

The CGIAR was set up in 1971 by the UN Food and Agriculture Organisation, the World Bank and the United Nations Development Programme to work towards increasing food production in the developing world by supporting agricultural research and training. It runs nine international centres, most of them concerned with crop research. Some concentrate on specific commodities such as cereals, rice and potatoes; some work on the problems of a specific ecological zone or region, such as dry areas or semi-arid tropics; and some work in the problems of a particular region such as specific livestock diseases in Africa.

The interests of the CGIAR reflect the results of studies quoted in the report which suggest that the world food problem is concentrated in the developing world, where the increase in population is outstripping any increase in food output, and that it is mainly one of crop production. According to one study quoted by Dr McCalla, 90% of the food energy eaten in developing countries comes from plants, 9% from animals and 1% from fish. Most of the plants eaten are cereals—wheat, rice, sorghum and millet—and potatoes, cassava and yams. Cereals are estimated to contribute 50-70% of total food energy.

There are two possible ways of easing the food problem of the developing countries, says Dr McCalla. Either they increase their food pro-

duction or there is an increase in the international trade of foodstuffs between them and the industrialised world. The former should improve food balances, but the latter might make matters worse.

In 1970, the world output of plant products was estimated at 3146 million tonnes of which 1208 million tonnes was cereals, accounting for 60% of total food energy and 73.5% of the world crop area. Trade in agricultural products, however, only amounted to 7% of global production, more than 50% being in cereals. A look at the origin and destination of trade shows that more than 75% of the volume of exports comes from developed countries and 80% goes to them.

Developing countries should, therefore, strive to increase their own food output. And the CGIAR research programmes, Dr McCalla concludes, might help them go some way towards achieving this. Based on four 'hazardous judgements'—the length of time a CGIAR centre has been open, the amount of research done on the commodity prior to the opening, the complexity of the research mandate of the centre and the imminence of important research results as anticipated by research workers—he estimates the likelihood of obtaining output increasing research within five to ten years (see table). The most promising crops appear to be maize and triticale, both under study in Mexico.

Judy Redfearn

Research centre	Research programme	Probability of output-increasing research		Research centre	Research programme	Probability of output-increasing research	
		0-5 years	5-10 years			0-5 years	5-10 years
CIMMYT (Mexico) Global mandate	Wheat	m	h	CIAT (Colombia) Regional and ecological Latin America tropics	Cassava	m-h	h
	Maize	h	h		Field beans	m-h	h
	Triticale	h	h		Livestock (forage)	1-m	m
IRRI (Philippines) Global mandate	Rice	m	h	IITA (Nigeria) Regional-tropical low altitude tropics	Farming systems	m-h	h
	Cropping systems (rice)	1	m		Roots and tubers	m	m-h
					Grain legumes	m	m-h
ICRISAT (India) Global and ecological zone mandate: semi-arid tropics	Sorghum	1-m	m	CIP (Peru) Global	Potatoes	m-h	h
	Millet	1-m	m		Livestock (systems)	1	1-m
	Pigeon pea and chick pea	1-m	m	ILCA (Ethiopia) Regional	Livestock (animal disease control)	1-m	m-h
	Ground nuts	1-m	m				
ICARDA (Syria, Lebanon, Iran) Ecological zone arid areas	Farming systems	m	m	ILRAD (Kenya) Regional			
	Barley	1-m	m				
	Lentils	1	1-m				
	Broad beans	1	1-m				
	Farming systems	1	1				

1—low m—medium h—high

CIAT—Centro Internacional de Agricultura Tropical CIMMYT—Centro Internacional de Mejoramiento de Maiz y Trigo CIP—Centro Internacional de la Papa ICARDA—International Centre for Agricultural Research in the Dry Areas ICRISAT—International Crops Research Institute IITA—International Institute of Tropical Agriculture ILCA—International Livestock Centre for Africa ILRAD—International Laboratory for Research on Animal Diseases IRRI—International Rice Research Institute.

Spain has forgotten her scientists

The politicians of newly democratic Spain are delaying urgent decisions on the future of science, writes **Pedro Puigdomenech**

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TWO and a half years have elapsed since the death of General Franco and the end of almost forty years of dictatorial rule over Spain. The country is making a transition to a democratic political system in the middle of an international economic crisis, and one that hits Spain to a greater measure than other western countries. This means that the process of political reform is accompanied by reorganisation of the economy.

In the last ten years research and development in Spain was kept around 0.3% of the gross national product (GNP). This figure is below the minimum of 1% recommended by OECD and puts Spain, with Greece and Portugal, far behind the other OECD countries. Spain is now spending more (0.4% of GNP) on the importation of foreign technology than in research. This is rather serious for the future of a country which claims to be the tenth industrial power of the world.

Part of the problem resides in the fact that most Spanish industries are either too small to afford any competitive research or are dominated by foreign companies that prefer to do their research elsewhere. This means, for instance, that industry offers very

few opportunities for science graduates. So students and professors often feel that their efforts are useless; and all this in a society which cares very little about the problems of university training.

Another problem is the organisation of Spanish research which is mainly carried out under the auspices of state bodies such as the Consejo Superior de Investigaciones Científicas (CSIC) and only to a minor extent in the Universities. These institutions are so marked by the practices of the previous regime that many people think that an increase in funding would be useless unless it is accompanied by radical reforms. In the process of political and economic reorganisation now under way Spain has the unique opportunity of being able to create a new science policy which is in agreement with the economic development and the future needs of the country.

The CSIC was founded at the end of the Spanish Civil War (1939) in order to "reconstruct the unity of science broken by rationalism". During the 40 years of its existence the CSIC developed a huge bureaucratic system filled with people mostly nominated for political reasons and without the par-

ticipation of scientists. So it can be understood why the first reform of CSIC was to abolish its whole internal organisation.

Universities are overcrowded, after a boom in the number of students over the last ten years. This has imposed a large teaching load on the professors, and is one of the reasons why Spanish Universities are very far from doing the amount of research necessary to offer proper training facilities.

Many problems are common to both the CSIC and universities, and the lack of an adequate financing is one of them. The CSIC budget increased in the last years at a rate just comparable to inflation, but as salaries rose more quickly, staff expenditures corresponded to 85% of its budget in 1975. Almost nothing is left for running costs. The problem is worse in universities where only the number of students and professors are normally taken into account in the distribution of credits. There is in theory a fund for research in universities (FIU) but it is distributed regardless of the research being done in the departments. The best one can expect is that the FIU will be spent on the students practical courses. Normally research in universities serves only to create PhDs; research work just stops after it.

Universities and the CSIC have a similar system of staff selection. This is a peculiar system of nationwide contest called "oposiciones". It consists of a series of examinations, written, oral and practical. In the university they emphasise breadth of knowledge, so militating against specialised people. Research experience hardly favours a candidate at all. In any case the result is normally known in advance or can be forecast by looking at the composition of the jury.

Appointments to the CSIC involve somewhat different criteria but there the question is that staff positions are very rare. Scientific staff in CSIC has increased less than 10% in the last six years. So the average age of research staff—47 in 1971—has been increasing since then.

The winners of "oposiciones" are considered to be civil servants. There is no control at all on their activity but no possibility of a research career in front of them. In contrast a high proportion of the research (100% in certain laboratories and departments) is done by staff 'under contract'. These contracts are for one year which means that most of the staff starts each year without a contract and will begin to be paid perhaps four months later. This situation can hardly be said to encourage research.

With very few exceptions, both CSIC institutes and University departments are far too small to be viable. The

Camera Press

average number of posts in the CSIC institutes is 7 with very often more than 7 different lines of research being followed. More than 50 institutes have three positions or less. University departments have normally only one full professor and therefore no stable research groups of viable size. At the same time administrative work is multiplied increasing the bureaucratisation of the staff.

Finally most of research institutes are in Madrid, where 70% of the CSIC staff are concentrated. This is especially serious for Spain where most of the industry is in two areas, Catalonia and the Basque Country. While only 5% of the CSIC research staff is in Catalonia, not a single research centre exists in the Basque Country; even the university has only recently been founded. It is not surprising then that research in Spain has no link with industry.

To coordinate the science policy in Spain an 'Advisory Commission for Scientific and Technical Research to the Prime Minister' was created in 1958. Its main function in recent years has been to distribute a fund (Fondo Nacional para el Desarrollo de la Investigación Científica y Técnica) that has been the only source of credit for research groups. This fund has the big advantage that credits are distributed to specific research projects that can last from one to four years. But, because of the poverty of the institutes and departments, everything—from salaries to running expenditures—is paid from these grants. This, plus the political desire to satisfy everyone, means that the fund is split into a large number of grants of insufficient size for the work to be undertaken.

For instance the latest available data (1975) show that 60% of the fund was divided among nearly 400 projects, totally unrelated one to another, to which less than 5 million pesetas (\$60,000) were attributed for 4 years. Only 80 projects got more than this figure and they were limited to a small number of laboratories mostly in Madrid. The amount of credit is unrelated to the research topic and there is a widespread feeling that the credits received are independent of the quality of the proposed project.

However, it is especially difficult to organise an objective system of grant distribution in such a small scientific community as there is frequently only one specialist in any particular subject. This causes difficulties quite apart from the tendency to favour friends which some people claim to be the typical Spanish way of taking decisions. This is the reason why it has been proposed that foreign referees should be asked to take part, at least in grant committees.

Another important source of funds

now declining was a programme of fellowships designed to train research workers (Plan de Formación de Personal Investigador) that included financial help to laboratories. This has resulted in a large number of trained people, many with experience in laboratories abroad, who have not been able to find positions in the CSIC or industry. Therefore Spain has now hundreds of well trained research people either underemployed in Spain or working abroad (it has been calculated that 75% of Spanish physicists working in research are abroad).

The change to a more democratic system of government has led to great hope in the Spanish scientific community. It should mean the end of an arbitrary, antiscientific system. In the last two years new ideas and proposals have appeared and, for the first time, they are openly discussed. Commissions of scientific planning have been created in Parliament and a restricted 'Cabinet for Research and Development' has met for the first time in many years.

Some reforms have already begun. In January 1977 the whole bureaucratic system of CSIC was abolished—although the CSIC still exists in name—and a new Statute issued in January 1978. It includes a Scientific Council with members elected by scientists (60%) and a system for selection of directors of institutes in which the staff of institutes takes part. Long-term projects have been announced—such as a General Law for Scientific and Technological Research and a Law for Universities.

Nevertheless, the main decisions have not been taken and the state of research is very uncertain in the near future. A new team has been appointed for direction of CSIC, but it is the third in two years. The Advisory Commission, that may include some scientists from the first time, is preparing a National Plan for Research. Planning is essential for a country with limited financial resources but it is dangerous if it does not take into account the state of laboratories and the experience existing in them. However, the fund this Commission administers may disappear; and it was the main source of credits for research. Autonomous governments have been appointed in several parts of Spain, beginning with Catalonia and the Basque Country. A congress of Catalan scientists asked recently for research to be put under the control of autonomous authorities, but such decisions are being taken very slowly.

There are several explanations for the delay that may cast a shadow on the scientists' hopes. All the reforms are being proposed in the midst of a deep economic crisis, and when there are other urgent political problems.

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Above: the science faculty of the 750-year-old University of Salamanca. Opposite: overcrowding at the same University.

People in office do not seem to consider science as an urgent matter, and therefore very few things have changed. Opposition parties share the responsibility for this because they include only vague proposals for research in their political programmes. Among the peculiarities of the Spanish approach to democracy is the fact that people who were in some of the key posts in the preceding regime remain there. Therefore many people doubt their ability to introduce the necessary reforms.

Hundreds of Spanish scientists are now waiting for the decisions to be taken in the next few months. Some of them are abroad waiting to come back if there is a minimum of change to enable serious work to be done. Some of them live in a continuous state of frustration under the precarious conditions in Spain. They are prepared to take part in the construction of the new economic and political system, if they are given a chance. In 1971 an OECD report on Spanish science concluded: "No nation if it is not to jeopardise its national character, can afford to be dependent for long on creative scientific activity carried out elsewhere". In 1978 it is to be seen if the newly democratic Spain will accept the challenge. □

correspondence

Genetic engineering guidelines

SIR,—I was very sorry to read the article by Robert Walgate entitled 'Genetic engineering guidelines: Europe hopeful about collaboration' (1 June, page 331). This referred to a meeting of the ESF's Liaison Committee for Recombinant DNA Research about which no official information has been released to the press nor sought by your journal.

I regret especially the suggestion made in the article that the NIH is misleading the European members of the committee. Our relationships with the NIH and with the Canadian Medical Research Council are, and have always been, extremely frank and open and cooperation between us is excellent. The common spirit which has been created in this committee was in fact particularly in evidence at this meeting.

We have no grounds for believing that the NIH is contemplating only a minor relaxation of their guidelines. On the contrary, we have been in close and continuous contact with them and the preparation of the revisions has involved a series of discussions in which members of the committee have taken part.

I regret that this kind of article can only put at risk the good transatlantic relationships which we have been building up over the last few years.

Yours faithfully,

F. SCHNEIDER

European Science Foundation,
Strasbourg, France

Advantages of nuclear energy

SIR,—Between one and three people are likely to die per year in Britain in the next few decades as a result of the operations of our civil nuclear power programme. In round figures a coal miner a week dies from an accident, a coal miner a day dies from pneumoconiosis and several members of the public die every day as a result of breathing the fall-out from the burning of fossil fuels. Even though coal provides more than ten times the proportion of Britain's energy that is provided by nuclear power, the latter clearly has a big advantage.

It is true that far more people are frightened by the smaller but less familiar risks of nuclear power than by the larger but more familiar risks of coal burning. Accordingly, it is natural and indeed perhaps proper for a politician to be more concerned with the risks of nuclear power. Dead people don't have votes. Frightened people do.

It is however a matter of regret that the chairman of the Conservative Committee on Energy, Nigel Forman, MP should take so unbalanced a view of the facts (1 June, page 332). Thus he is afraid of the "legitimate doubts" of unspecified dangers from glassified wastes, of which satisfactory blocks in nickel alloy canisters have been made in both Britain and France, and which have been tested in Britain to levels of radiation damage corresponding to a million years

of storage. Less than a thousand years of storage would leave them no more active than a good uranium ore, but he cites with approval a Swedish suggestion that unprocessed fuel elements could be stored in copper or ceramic canisters. Probably they could, if put sufficiently far underground, but why should they be safer? After some hundreds of years they really would be worth exhuming by terrorists for the 1% or so of plutonium contained, which by then would be free enough of gamma-emitters to be processed in an unscreened laboratory without excessive radiation doses to the operators.

Extraction of the remaining traces of plutonium in the glassified blocks is at the practical limits of current technology and to process a hundred tons or so for the plutonium to make a bomb is unlikely ever to be attractive to amateurs. I agree with him that we "are still relatively low on the learning curve about the full potential of a safer, cheaper, alternative energy future", except that I would have said that we don't yet know whether it will ever be possible to make it cheaper or certain that it will ever be safer.

If, hopefully, effective alternatives do appear, they could be used together with nuclear power to conserve irreplaceable oil and coal, not to replace the safer and cleaner nuclear power.

Mr Forman states that a further eighteen months delay could be added without loss to the two years delay already incurred. How can he know this? Countries such as Japan with no oil and little coal are not going to rest their entire future existence on hopes of alternatives. They must build up a nuclear option capable of supplying their industries in case these hopes are not fulfilled. If THORP as well as the US refused to reprocess, they would have to do it themselves or turn to the USSR. Of course there is a risk—indeed almost a certainty—of proliferation of nuclear weapons to some extent whatever we do. The necessary data have all been published but I cannot see why the rate of proliferation should be increased if processing is done by large units in a few countries under close international control rather than separately in a large number of countries which may well refuse any control at all, or do the work in secret, as a result of what they see as a unilateral refusal by the US to implement the positive parts of the non-proliferation treaty.

The real danger before the world seems to me to be the risk that no adequate source of power may be available before the oil gets short. This is indeed the only shadow of rational explanation of the \$200,000 million being spent annually on armaments. If the alternatives don't work, there could come a time when military control of the Middle East could mean economic control of the world. It may be that even if developed at the maximum possible rate, nuclear power will still not be sufficient in time to avoid disaster. It may be that alternative supplies will already be ample to replace both uranium and coal, in which case two more generations of nuclear power stations will

be enough. But it will be really dangerous not to have the option.

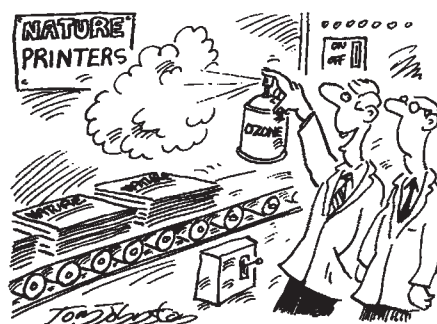
Yours faithfully,

J. H. FREMLIN

University of Birmingham, UK

Nature's ozone

SIR,—Working with electrical-discharge devices has made me familiar with the characteristic smell of ozone, which to me has both the "oily" and the "fresh air" qualities often attributed to it. I read about two dozen different science-oriented periodicals per month in the course of my work, but it is only when reading *Nature* that I detect this same smell. (I realise that the articles appearing in *Nature* are



"It's the only air-freshener that doesn't damage the ozone layer"

intended to be stimulating, but hopefully not electrically so.)

I would like to bring to your attention the question of whether ozone indeed is being generated, perhaps by some static phenomenon in the process of turning pages. Or is the smell merely characteristic of the paper or the printer's ink? If the latter case is true, it would be interesting to learn what compound in the periodical mimics the olfactory stimulus of the O_3 molecule.

Yours faithfully,

CHARLES M. CEGIELSKI

Chicago, Illinois, USA

Anti anti-'x' antibodies

SIR,—During the last year, I have noticed that scientists increasingly use the term anti-'x'-antibody both in publishing and lecturing to describe an antibody directed against substance 'x'.

It may be a matter of semantics, but as one can actually produce antibodies against antibodies (which anti-'x' already implies), this leads to confusion in nomenclature.

I, as a former scholar of ancient Greek, who now works in immunology, would like to suggest that authors and speakers should use the prefix "anti"—against only once when they describe antibodies against substance 'x', such as anti-'x'; anti "x" serum; "x"-antibodies, or antibodies directed against "x".

Yours faithfully,

UTE GRÖSCHEL-STEWART

Darmstadt, West Germany

news and views

FOR some weeks there have been rumours in the high energy physics community that parity violation was being seen in an electron-scattering experiment at Stanford, California. The significance is that electron scattering is the canonical example of an electromagnetic interaction (it is electromagnetism that holds the electrons in the atoms for instance), hence this is a new example of parity violation. The group concerned have now officially announced this to be the case. This result fulfils exactly the predictions of the Weinberg-Salam unified model of the weak and electromagnetic interactions.

The 'parity' involved is a parity between left and right. In a mirror left and right are interchanged: write with your right hand—behind the mirror you see a left-hander. But as left-handers also exist in the real world there is no way of distinguishing from a picture of a left-hander whether he is 'real' or a mirror image. This indistinguishability is called 'parity' conservation.

One of the cultural shocks of our generation was the discovery in 1957 that in the subatomic world one could distinguish right from left absolutely. The key is the weak interaction (the force of nature responsible for radioactivity). Radioactive decays emit neutrinos. These are massless uncharged ghostly particles whose only obvious property is that as they travel through space at the speed of light, they corkscrew. The interesting feature is that they always spin anticlockwise (left-handed corkscrew). Viewed in a mirror one would see a neutrino corkscrewing in a right-handed sense. Such neutrinos do not exist in the real world. Hence the real and mirror worlds can be distinguished. This is the famous violation of parity.

Before this discovery Pauli is supposed to have said that he could not believe that God was a weak left-hander. Afterwards he came to terms with it and said that the mystery was not why he was a weak left-hander but why he was not left-handed everywhere. No evidence for parity violation has ever been found other than in the weak interaction—until this new result.

The facility at Stanford can produce high energy electrons that corkscrew

New source of parity violation

from F. E. Close

left-handed (like a neutrino) or right-handed. It is because both of these configurations exist for the electron that parity is conserved in electromagnetic interactions whereas parity is violated in neutrino interactions as a result of the absence of right-handed neutrinos.

Now fire these electrons at a proton. It was found that the left-handed electrons have a greater tendency to interact with the proton than do right-handed ones. The left-handed excess is only one event in every 10,000 or so, but this is a large effect within the sensitivity of the experiments. Hence we have a new way of distinguishing the real from the mirror world—there are both left-handed and right-handed electrons, but it is the former that prefer to interact in the real world.

The theoretical significance of this discovery is that it had been correctly predicted both that left-handed should win and by how much. The prediction comes from the Weinberg and Salam unified model of weak and electromagnetic interactions about which much has been written recently (see *News and Views* 264, 505; 1976; *News and Views* 273, 98, 706; 1978).

There has been much recent evidence supporting this idea that the weak and electromagnetic interactions are but two faces of the same coin ('unified'). If true this innovation parallels Maxwell's nineteenth century unification of electricity and magnetism into modern electromagnetism. As parity is violated in weak interactions it is not surprising that the unification of the weak with the electromagnetic interaction would cause one to predict that interesting parity violations should be found in processes that are 'electromagnetic'. The Stanford electron scattering is a particular example of such a process and other examples include certain phenomena in atomic physics (see *Nature* 264, 528; 1976). While experiments in atomic physics are still continuing, we now have the first clear evidence supporting this 'new parity violation' and, moreover, it seems to be precisely as the model predicted.

The source of the parity violation is as follows. In standard electromag-

netic theory the electron and proton interact by exchanging photons. This process conserves parity, hence is equally probable for left and right-handed electrons. In standard weak interaction theory neutrinos turn into electrons and interact with protons by exchanging electrically charged W bosons. These are very massive and will be actively searched for at the next generation of accelerators. The left-handedness of the neutrino causes parity to be violated here.

In the unified theory of weak and electromagnetic interactions both of the above naturally occur. In addition, two new types of effect are predicted. In the first of these, neutrinos can remain neutrinos and interact with protons by exchanging a neutral W boson (actually this is called the Z^0). This also is very massive and on the list of the searchers. This interaction violates parity because the neutrino is left-handed. Such an interaction (weak neutral current) was first seen in 1973 and seems to violate parity (though some technical assumptions have to be made in order to claim that parity violation has really been seen here).

Just as the theory predicts that a Z^0 can couple to the (left-handed) neutrino, so it predicts that the Z^0 can couple to electrons. This is the second effect referred to above.

You may be anticipating that the Z^0 couples only to left-handed electrons; this is not quite correct. It is the case, however, that it does not couple equally to left and right-handed electrons. The actual preference for the one or the other is expressed in terms of a parameter called the Weinberg angle with a value (deduced from other data) $\sin^2 \theta \sim 1/4$.

Hence when an electron scatters from a proton it does so not only by the familiar photon exchange of electromagnetism (equally in right and left-handed modes), but also, albeit rarely, by the Z^0 exchange contribution ('weak neutral current'). This is predicted to favour left-handed electrons if $\sin^2 \theta \sim 1/4$.

It is this observation of the left-handed excess which has provided the first evidence that the neutral current couples to electrons, and the first direct proof that it violates parity. The size and left-handedness of the parity violation seems to point clearly to the Weinberg-Salam model as being the correct model for unifying the weak and electromagnetic interactions. □

F. E. Close is at the Rutherford Laboratory.

Dissecting H-2 restriction

from Rod Langman

THE phenomenon of H-2 restriction is barely four years old, but already it is at the centre of a major shift in immunological thinking. A recent workshop meeting* brought together a small group representing the major experimental systems and theoretical viewpoints concerned with H-2-restricted cytotoxic T cells.

The haemopoietic radiation chimaera has provided one of the most powerful approaches to unravelling the mechanisms whereby T^K -cells become restricted to the 'self' H-2 or R (restricting) antigen. For example, R. Zinkernagel (Scripps Clinic) and M. Bevan (Massachusetts Institute of Technology) showed that it was the H-2K and H-2D R-antigens on radioresistant host cells that determined the 'self' specificities. A typical experiment would be to repopulate an irradiated $H-2^b$ mouse with $(H-2^b \times H-2^d)$ F_1 stem cells and show that only $H-2^b$ -restricted T^K responses could be obtained. As Zinkernagel then went on to show by thymic grafting, it is the H-2 type of the radioresistant thymic epithelium that determines the anti-'self' R-specificities.

I. Weissman (Stanford University) reported on the distribution of H-2 IA, H-2 IJ and H-2 K/D antigens in thymus as shown by indirect immunofluorescence, using alloantisera that had been carefully screened for antiviral activity. While the majority of epithelial reticular cells throughout the thymus stained with anti-H-2 IA sera, the anti-H-2K and D sera stained in patches that were located primarily in the medullary and deep cortical zones, and the anti-H-2 IJ sera stained a few patches in the cortical-medullary junction. The significance of this predominant expression of H-2 IA antigens was not immediately apparent although the differential distribution of IA, K/D and IJ antigens strongly supports the idea that there are specialised restricting cells in the thymic epithelium.

There are several examples of H-2K or H-2D allele-specific control of T^K response to virus and TNP-modified cells. S. Burakoff (Harvard University) showed that the low degree of cross reactivity found in $H-2^k$ anti-TNP- $H-2^k$ responses could be made highly cross-reactive in $(H-2^k \times H-2^b)$ F_1 animals and mapped to the H-2K^b-IA^b region. Using radiation chimaeras of the type $H-2^k \rightarrow (H-2^k \times H-2^b)$ F_1 it was found that TNP- $H-2^k$ cells induced a highly cross-reactive response. This is an important experiment because it implies that the cross-reactivity pattern is determined

by the restriction phenotype, not the genotype, of the responding cells. Although it has often been argued that response patterns could be determined by the differential association of antigens with the restricting antigens, in these experiments TNP- $H-2^k$ cells were a constant source of stimulating antigen. Therefore, the response pattern in the chimaera must have been determined by the restriction specificities in the responding T cell population.

Zinkernagel then reported on an example of H-2K^k-determined dominant unresponsiveness to H-2D^b-restricted anti-vaccinia virus. In these experiments $H-2^b$ mice make a good response to H-2D^b-vaccinia while $(H-2^k \times H-2^b)$ F_1 mice respond poorly, but an F_1 between $H-2^b$ and B10.BYR ($H-2K^d$ IA^kD^k) makes a good response. The obvious possibility raised in any case of dominant unresponsiveness is that of cross-reactive self tolerance; in this example H-2K^k-self would supposedly mimic H-2D^b-vaccinia. This was ruled out by showing that $(H-2^k \times H-2^b)$ F_1 spleen cells could respond to H-2D^b-vaccinia when immunised by adoptive transfer to irradiated and infected mice which lacked the H-2K^k antigen, for example, $H-2^b$ or $(H-2^b \times B10.BYR)$ F_1 mice. Furthermore, chimaeras of the type $(H-2^b \times H-2^k)$ $F_1 \rightarrow H-2^b$ also made a good response to H-2D^b-vaccinia, again emphasising the conversion of non-responder (F_1 cells in this case) to responder phenotype by making them become restricted to responder ($H-2^b$) thymic R-antigens. Having ruled out the possibility of cross-reactive self tolerance as an explanation of dominant unresponsiveness in these experiments it becomes necessary to consider what might be termed the 'immuno-dominance' of H-2K^k-vaccinia over H-2D^b-vaccinia. When interpreting this in terms of the various models of H-2 restriction M. Cohn (Salk Institute) argued for a two receptor model in which the affinity of anti-X (vaccinia) is low and the affinity of anti-R (K^k) is high compared with anti-R (D^b), thus favouring the induction of a K^k -vaccinia response over D^b -vaccinia. However, when the results of H-Y (male-specific antigen) experiments were reported, it was clear that $(H-2^b \times H-2^k)$ F_1 females responded to H-2D^k-H-Y in preference to H-2D^b or H-2K^k-H-Y, and this argued against a simple anti-R affinity interpretation. In general, no model of H-2 restriction was presented that could account for all of the data in a fully satisfying way.

During the discussion of restricted killing to the H-Y antigen it became

obvious that there were some major disagreements over which strain combinations could produce anti-H-Y responses. H. von Boehmer (Basel Institute for Immunology) found that $H-2^b$ mice were the only homozygous strain to respond, along with the F_1 hybrids between $H-2^b$ and all other strains. On the other hand, E. Simpson (Clinical Research Centre, Harrow) found additional cases where the F_1 between two non-responders produced a response to H-Y. Although the precise basis for these discrepancies could not be nailed down, the most likely difference seems to be in the *in vivo* immunisation protocol. The resolution of these discrepancies is of considerable theoretical importance because von Boehmer, Haas and Jerne have recently proposed a theory of H-2 restriction which must almost certainly be true if the von Boehmer H-Y results are correct, and equally untrue if the Simpson H-Y results are correct. The theory assumes two receptors that are initially identical and can be selected as anti-self R, then one receptor is allowed to mutate and generate the anti-X repertoire while the other receptor remains constant as anti-R (self). Since von Boehmer finds that H-Y unresponsiveness is dependent on the presence of H-2 IA^b on the responding cells, and is also obviously required as an R specificity, the argument is that anti-H-Y can only be generated when anti-IA^b is used as the starting anti-X specificity.

Theoretical discussions centred on the origin and nature of the T^K receptor(s). None of the many theories has yet been ruled out, and it seemed that every participant had a different theory. There was a lengthy discussion on the use and meaning of the popular term 'altered-self' which concluded with general agreement that the term NAD (for New Antigenic Determinant) was more precise and should be used whenever possible. An NAD was defined as an antigenic determinant that is present when R and X molecules form a complex, but is absent when these are separate. It was also clear that the term 'dual-recognition' needed clarification as it did not accurately describe all of the non-NAD models, as is often implied. The consensus was that particular models should be referred to rather than vaguely alluding to a class of models. It is perhaps time for these various models to be collected and subjected to a single review.

*The Armand Hammer Cancer Workshop on H-2 Restriction was held at The Salk Institute, San Diego, from 30 May-2 June.

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The analysis of cellular and antigenic requirements for the induction of T^K responses was a major issue of investigation, although theoretical discussions failed to deal with the question in detail. G. Shearer (National Institutes of Health) presented data to show that macrophage depletion of the responding and TNP-derivatised stimulating cells abrogated the anti-TNP T^K response. While allogeneic macrophages could restore the response, they did not affect the specificity of restriction. This is in contrast to the proliferative responses which required H-2I compatible macrophages that have been TNP-coupled. P. Doherty (Wistar Clinic) described an intricate though elegant experiment to test whether T^K induction required H-2I compatible antigen presentation. In outline, the experiment involved removing H-2^k reactive cells from H-2^b TDL by negative selection in F_1 hosts then adoptively immunising the TDL in K^k , I^k , D^b irradiated and vaccinia-infected hosts. A good K^b vaccinia response was obtained arguing that the induction of T^K could not require the participation of H-2I^k (incompatible) vaccinia antigen complexes. In a quick assay around the table, nobody could produce any evidence that H-2I-restricted T cells were required in the induction of H-2K/D-restricted T^K , although there seemed to be ample evidence that H-2I-incompatible radiation chimaeras of the $H-2^a \rightarrow H-2^d$ type were essentially unable to mount a successful T^K response. Taking this in conjunction with the observation of high levels of expression of H-2IA antigens on the thymic epithelium, many people were led to wonder if IA has some crucial role in T-cell differentiation rather than being simply the restricting antigen for helper T cells as is popularly believed. □

New evidence for an explosion in galaxy M82

from Geoffrey A. Cottrell

A SUFFICIENTLY violent explosion deep in the nucleus of a galaxy can expel hot gas right out of the galaxy. If such an explosion occurs in the centre of a disk galaxy, the ejection is most likely to take place in two oppositely directed and symmetrical beams, channelled along the rotation axis of the galaxy. This theory can be tested by making spectroscopic observations of the Doppler shifted line radiation emitted by the outflowing gas, from which one

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Accelerated soil erosion

from A. J. Low

THE loss of soil by erosion induced by man's activities is now a serious and increasing problem. More land is brought into cultivation every year to support a growing population, but the overall loss from erosion is greater than the gain. A Workshop on the assessment of erosion in Europe and the USA was held recently in Ghent.*

The urgent need today is for an assessment of soil erosion on a world scale. The extent of soil erosion can be predicted by applying the Universal Soil Loss Equation (USLE) which takes into account the erosivity of the rain and the erodibility of the soil. The rainfall factor is normally the most difficult to obtain and this has limited its use to countries like the USA. H. M. J. Arnoldus of FAO submitted to the Workshop an index he has devised providing a rapid approximation of the rainfall factor which could greatly speed up the production of small scale maps in developing countries.

A vivid account of soil erosion control measures in China today was given by R. Dudal (FAO, Rome). For centuries China has suffered from severe soil erosion with consequent silting of the rivers leading to disastrous floods. In China (as elsewhere) establishment of forests in the denuded landscape, particularly in the

watersheds is the major method for reducing erosion, with grassland where forestry is not possible. Forestry and agriculture are closely integrated in China today. The Chinese are attempting to reduce erosion tolerances to zero by reducing slopes and levelling land on a vast scale mostly by hand labour. In the USA soil loss tolerance has been set at about 5 ton/acre/year but there is little economic incentive to attain this and it is frequently ignored. The Chinese with 22% of the world's population and 7% of the world's agricultural land regard the soil as a vital resource to be husbanded at all cost.

In Europe France occupies the first rank in agriculture. But due to excessive deforestation, and intensive cropping, particularly through hasty land unification and unsuitable farming techniques, soil erosion has been accelerated and river flow regimes altered, leading to severe flooding. The key to slowing down erosion is the protection of watersheds by afforestation leading to regularisation of mountain torrents. Such measures are now standard practice in France where particular attention is being paid to fragile morpho-climatic zones.

By and large we know how to reduce soil erosion: the problem is how to implement or enforce the necessary procedures. □

* Held on 27 February—3 March at the University of Ghent. The Proceedings will be published by John Wiley.

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can deduce the large scale motions of the gas. Essential to the explosion model is the occurrence of a purely radial outflow; it is over this key feature that the controversy about whether a gigantic explosion has arisen in the nearby peculiar galaxy M82 has hinged.

Fifteen years ago, Lynds and Sandage observed, in M82, a filamentary structure which extends above and below the plane of the galaxy, revealing a radial velocity gradient which suggested that matter was being expelled from the nucleus along the minor axes with peak velocities of the order of $1,000 \text{ km s}^{-1}$. However, some later observations showed that the narrow ($\sim 10 \text{ \AA}$) $H\alpha$ line and the continuum emission from the filaments were highly ($\sim 30\%$) polarised. This indicated that the radiation from the filaments was not intrinsic, but was more likely to be

radiation from a bright nucleus, scattered by dust grains above and below the plane of M82. If the grains were moving purely radially away from the central source, as required by the explosion model, then the scattered light should be redshifted on both sides of the plane. But the observations showed that the $H\alpha$ was redshifted on the north side of M82 and blueshifted on the south, thus creating serious difficulties for the model. In a paper by Solinger *et al.* (*Astrophys. J.* **211**, 707; 1977), the explosion hypothesis was discarded in favour of an interaction model—namely that M82 is moving through an intergalactic dust cloud in the M81 group, and is thereby sweeping up gas which is triggering star formation in the galactic disk. In this model, the red and blue shifts are explainable by a motion (in the plane of the sky) of the source of scattered

light (M82 itself) with respect to a stationary scattering medium (the dust cloud). However, recent high resolution radio observations of the 21-cm neutral hydrogen line emission surrounding M82 (Cottrell, *Mon. Not. R. astr. Soc.* **178**, 577; 1977) show apparent rolling motions of the neutral gas around the major axis of M82. These results are not compatible with the model of Solinger *et al.*; that is, M82 is not moving through a stationary scattering medium. Furthermore, M82 is connected to M81 by a neutral hydrogen bridge which, at either end, merges into the galaxies with radial velocities close to their optical radial velocities. The existence of this bridge strongly suggests that a gravitational tidal interaction occurred between M81 and M82 about 10^2 Myr ago.

David Axon and Keith Taylor (see page 37 of this issue of *Nature*), using the Isaac Newton Telescope, have now discovered a new and important fact. They have found that the H α and [NII] emission lines from the southern filaments are split. The magnitude of the splitting is considerable ($\sim 250 \text{ km s}^{-1}$) and, even more interestingly, the spectra form complete and hollow ellipses. These observations cannot be explained by the model of Solinger *et al.* (indeed by any scattering model) unless there is a very special scattering geometry. Furthermore, the linewidths measured by Axon and Taylor are significantly narrower than those observed in the direction of the presumed scattering source; this fact is also hard to reconcile with any scattering model. So we are now forced to look again at an explosion model and an intrinsic emission mechanism in the filaments. Axon and Taylor admit the difficulty in reconciling the intrinsic emission mechanism with the observed equivalence of the H α and continuum polarisations but suggest that the problem may be circumvented if the intrinsic emission from the filaments is superimposed on a weaker but highly polarised scattered component. The simplest interpretation of the new observations is that the filaments are expanding away from the nucleus on the surface of a cone. This gives a natural explanation of the hollow elliptical spectra.

A more complete model must await further observations, particularly those which can be made on the northern filaments (in order to test the symmetry of a possible 'double cone' outflow of matter) and also additional polarisation emission line measurements over a wider region of the filaments. The ejection velocity is $\sim 400 \text{ km s}^{-1}$, implying a time since the explosion of about 10 Myr. This explosion is one remarkable event in the history of M82. But

there was an earlier remarkable event in its history, a violent tidal interaction about 10^2 Myr ago. One wonders if these two events are not causally

related? There is still much to be learned about this most fascinating and puzzling galaxy. □

HLA-D locus and susceptibility to disease

from R. T. D. Oliver

RHEUMATOID arthritis (Stastny *New Engl. J. Med.* **298**, 869; 1978) and Goodpasture's syndrome (Rees *et al. Lancet*, **i**, 996; 1978) have now been added to the list of diseases where a highly significant increased incidence of specific HLA-DRw antigen has been associated with disease susceptibility. Stastny reported a 70% incidence of HLA-DRw4 among 54 patients with rheumatoid arthritis compared with a 28% frequency in a control population. Rees *et al.* reported 88% incidence of DRw2 in a series of 17 patients with Goodpasture's syndrome compared with 32% in a control population. Though the number of patients tested in this latter study was small and requires independent confirmation the difference was highly statistically significant. Confirmation of the association of DRw4 with rheumatoid arthritis has come from Roitt *et al.* (*Lancet*, **i**, 990; 1978) who also pointed out that the association was strongest in patients with the most aggressive form of the disease, where 94% of patients were DRw4 positive. The importance of these findings can be considered from four points of view: their contribution to the understanding of the genetics of the HLA complex in man; their relevance to the understanding of the pathogenesis of these diseases; their clinical significance in terms of diagnosis and treatment of the patient; and their evolutionary significance.

Since 1967 the proliferation of diseases, mainly of undetermined aetiology, which have shown associations with a particular HLA antigen or group of antigens has been quite staggering. It is known that genetic information controlling the expression of the gene products of the four loci (A, B, C and D) of the HLA region is on chromosome 6. However in addition loci controlling complement factors C'2, C'4 and Bf as well as erythrocyte antigens Rodgers (Rg) and Chido (Ch) and several enzymes including phosphoglucomutase 3 (PGM3) and glyoxalase (GL) have been mapped to the same segment of chromosome 6. This multiplicity of loci and the fact that the phenomenon of linkage disequilibrium has caused certain alleles at the different

loci to remain associated in unrelated individuals, means that it is not certain that the disease association is due to the specific antigen reported or alleles of a closely linked locus. This problem is clearly demonstrated by the results of HLA typing of patients with a multiple sclerosis and coeliac disease. Both these diseases showed a significantly increased frequency of specific HLA-A and B locus antigen but when typing for D locus antigens became available there was an even stronger association with a specific D locus antigen. The studies of Stastny and Rees are the first to report a clear HLA-DRw antigen and disease association without a significant disturbance of HLA-A and B locus antigen frequency. The fact that both these diseases (rheumatoid arthritis and Goodpasture's syndrome) have clearly defined abnormalities of immune response (rheumatoid factor/antibodies to glomerular basement membrane) provides further support for the idea that there are genes regulating immune response associated with the HLA-D locus. Most of the evidence for the existence of HLA linked genes regulating immune response in man is speculative due to the lack of large enough studies of immune response testing in families. The most convincing evidence from testing in normal unrelated individuals comes from Japan where low response to tetanus toxoid has been associated with DwDHO, an antigen so far only detected in Japanese (Sasazuki *et al. Nature* **272**, 359; 1978). Applying such an approach to families with HLA-D associated diseases using a wide variety of antigens is going to be the only way to establish positively the antigenic basis of the aberrant immune response gene and prove that it is involved in the pathogenesis of the disease. In the meantime, in the absence of techniques to determine immune response gene polymorphism, HLA-D locus typing will remain the best indirect approach to investigating immune response gene influence in man.

Typing techniques

Typing for D locus antigens has not yet reached the level of precision that is currently available for typing of HLA-A, B or C locus antigens. There is still some controversy whether the

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two techniques used—homozygous typing cells which define Dw antigens, and B cell serology which defines DRw antigens—are detecting products of the same locus or closely linked loci determining separate antigenic sites which could possibly be on the same molecule. The data produced by the recent 7th International Histocompatibility Workshop (Histocompatibility Testing, 1977, (ed. Bodmer *et al.*) Copenhagen, Munksgaard, in press; summarised in *News and Views* 270, 664; 1978) did not provide a clear answer but favoured the unitary hypothesis because concordance between these typing techniques was as good or better than the reproducibility of the tests, and there was supportive evidence from biochemical and other immunological assays in agreement with this hypothesis. There is no doubt that the technology of DRw typing is simpler and less time-consuming than MLC typing for Dw alleles and thus more suitable for large scale HLA and disease studies. However, even in mice it is by no means certain that MLC-defined and B cell serology-defined antigens of the H-2I region (murine homologue of HLA-D locus) are the same. Furthermore, the greater complexity of this region in mice suggests that the HLA-D locus will turn out to be even more complex than at present defined.

Turning to the relevance of Stastny's and Rees *et al.*'s observations to the pathogenesis of rheumatoid arthritis and Goodpasture's syndrome, it is clear that at present these observations add little more than already contributed by the demonstration of rheumatoid factor or the antiglomerular basement membrane antibody. However, they do add another fact which has to be considered in the study of the pathogenesis of these diseases. In particular it means that it will no longer be adequate to use random unrelated groups of individuals as controls for this study. This is highlighted by experiments in rats exposed to mercuric chloride. Antiglomerular basement membrane antibodies only developed in those rats with a specific histocompatibility phenotype (Druet *et al.*, *Eur. J. Immun.* 7, 348; 1977). It is obvious that these findings make it very important to re-evaluate previous equivocal studies on effects of postulated environmental agents such as mycoplasma infection in rheumatoid arthritis and Gram-negative bacterial organisms in ankylosing spondylitis by studying normal and diseased individuals with the same HLA phenotype.

Diagnostic typing

From the clinical point of view HLA typing cannot yet be considered of diagnostic value. The great majority of

individuals possessing disease-associated HLA-antigens do not have the disease. Even for ankylosing spondylitis, the disease with the closest correlation with a specific HLA antigen, 85% of normal unselected individuals with B27 do not have the clinical signs and symptoms of ankylosing spondylitis. Nevertheless it is apparent that knowledge of HLA phenotype may lead to a reclassification of currently defined diseases. Already there is some indication that HLA phenotype is of help in differentiating subgroups of patients with diabetes mellitus and myasthenia gravis. In addition the results from HLA studies may be of use in predicting patients with a more severe form of disease as has been suggested from the studies in rheumatoid arthritis and possibly also in coeliac disease (Kumar & Oliver, in preparation) and asthma associated with nasal polyposis (Moloney & Oliver, in preparation). This may also be the case in malignant disease where several studies have reported more favourable prognosis associated with a particular HLA antigen (for review, see Oliver, *Br. J. Hosp. Med.*, 1977), though to date none of these studies has involved HLA-D typing.

Evolutionary significance

The evolutionary significance of these observations has been little investigated. It is paradoxical that the HLA antigens most highly associated with disease susceptibility are those with the highest frequency in the normal population. However, most of these diseases are relatively rare chronic diseases, usually with onset after puberty and rarely leading to death until after the normal period of adult fertility. This might suggest that these genes became predominant under the influence of selective pressures which are no longer active and the currently associated disease is a late by-product of what was originally a beneficial trait.

This phenomenon is most clearly demonstrated by the diseases associated with HLA A1/B8/DW3. This is the most common haplotype in Caucasian populations, although it is virtually absent from mongoloid and negroid populations. The same antigens do occur in other populations but show different associations (for example, A1/B17 in Asian Indians and AW30/B8 in Zambian negroes). This observation makes it unlikely that a recent mutation is the explanation of linkage disequilibrium and is more compatible with the idea that these haplotypes have been associated with some evolutionary advantage. The diseases associated with A1/B8/DW3 haplotype (for example coeliac disease, Graves exophthalmic goitre, chronic active hepatitis, myas-

thenia gravis) all have an underlying immunological abnormality and were of little consequence during the pre-antibiotic era when infectious disease was a major cause of death in early life. So far there is no direct evidence that this haplotype has had any survival advantage during epidemics of infectious disease, although there are two suggestive clues—the demonstration that HLA/A1/B8 dialysis patients are more efficient at clearing rival antigen from blood during hepatitis B infection (Jungers *et al.* *Transplant. Proc.*, in the press) and the better chance of survival seen in patients with Hodgkin's disease and acute myeloid leukaemia who were A1/B8. More definitive studies of HLA typing of susceptible and resistant populations in closed communities during infectious disease epidemics may provide more direct information on this problem. Suggestive evidence in support of this hypothesis has already been reported from a retrospective study of Dutch nationals living in a closed community in Surinam after surviving major epidemics of typhoid fever (van Rood *et al.* *Prog. Immun.* 3, 338; 1977).

In conclusion, there can be little doubt that study of HLA-D typing in human disease has already been a productive area of both fundamental and applied research, though much further work remains to be done. At present there is little justification for the cost of making HLA typing available on a service basis for diagnosis, though clearly it will not be long before this possibility has to be considered, particularly if immunotherapeutic approaches are developed to overcome the deficit of the abnormal immune response gene. □

Structural options

from D. Z. Staynov

THE appearance a decade ago of the complete sequences of histones H3 and H4 from different species confronted workers in the chromatin field with a vexatious puzzle, for the sequences were seen to have been subject of a degree of evolutionary conservation unparalleled in any other known type of protein. Cytochrome *c*, for example, is a highly specialised and conserved protein and histones H2A and H2B have in common with it a rate of change of 3 PAM (accepted point mutations per 100 residues per 10⁸ yr). By contrast the rate for H3 and H4 is only 0.1 PAM. The consequence is that in the millennia which have elapsed since plants and animals began to diverge, differences have

appeared in only five residues, all variants being non-allelic. Furthermore, the sequences of H3 and H4 have extensive similarities, which suggests a common evolutionary origin, with H3 perhaps derived from H4. This implies that evolution had already proceeded to the point at which all further mutations were lethal. A speculative explanation of all this runs that all the side chains of H3 and H4 were involved in either intra or intermolecular interactions, involving precise histone-histone or histone-DNA contacts, whereas the other histones have much of their surfaces freely exposed to the solvent. But this argument scarcely holds water, for other tightly packed structures, such as ribosomes, which are at least as compact as chromatin, do not show such evolutionary constraints.

A different explanation suggests itself, namely that the two histones must be capable of existing in two structurally distinct stable globular states. In a globular protein a considerable proportion of the residues are not critically involved in function or packing, and it is these which are subject to mutational changes. If, however, the chain were required to be capable of folding into a different globular structure, the number of mutable residues would decrease according to a second-power law. Perhaps then such a conformational adaptability is a property of H3 and H4 histones, the evolutionary process having slowed down sharply at the point at which the second conformation became a stable reality and started to be functionally utilised. Now we do indeed know that interphase chromatin as a whole must be able to exist in at least three different functional states. These are transcriptionally active, potentially active and inactive respectively, and a memory for each of them must survive throughout the mitotic cycle. What is not clear *a priori* is whether each state corresponds to a different conformation of the chromatin.

After the achievements between 1973 and 1975, which established by electron microscopy and nuclease digestion experiments the basic facts of a periodic structure in chromatin, it became clear that the bulk of interphase chromatin, as well as mitotic chromosomes, was organised into nucleosome-like structures. As regards the relation to transcription, electron microscope studies revealed that the transcriptionally active rRNA genes—transcribed in isolation in the nucleolus by their own polymerase—probably have no nucleosome structure, but for

single-copy structural genes results have been ambiguous. On the other hand, nuclease digestion experiments suggest that both rRNA genes and the structural genes possess a repeating structure, and immunochemically-assisted electron microscopy has given evidence that histones are to be found in association with transcriptionally active genes. DNA in nucleosomes, however, is tightly packed, and cannot serve as a template for DNA or RNA polymerase. Thus a relaxation or modification of the structure must at some point occur. Whatever the nature of this change, it must be associated with a low energy of activation and probably cooperativity within the gene. It can scarcely necessitate a dissociation of the strong electrostatically stabilised interactions of DNA with histones, and histone-histone interactions, modulated by a change in a single residue or by an interaction with a nonhistone protein, are better candidates. Moreover as has been shown by Camerini-Otero and Felsenfeld (*Nucleic Acids Res.* **4**, 1159; 1977), such a transition does not theoretically require high energy. This is perhaps a long preamble to a consideration of a recent paper by Gordon *et al.* (*Proc. natn. Sci. U.S.A.* **75**, 660; 1978), but it places its possible importance in perspective. On the basis of the hydrodynamic properties of isolated nucleosomes, free of the very lysine-rich histone, H1, they have found that changes of ionic strength can give rise to two highly cooperative, reversible structural transitions. These are centred at sodium ion concentrations of 1 and 10 mM, are not accompanied by any release of histones, and are exceedingly sharp. The transitions are rapid on the hydrodynamic time-scale, and are thus characterised by low activation energy, and can be prevented by introducing artificial covalent cross links between histones. The differences in frictional properties between the three states of the nucleosomes are very considerable, and the structural effects therefore sizeable. One can at this stage say little about the nature of the differences, but it seems likely that the existence of such stable states, notwithstanding the grossly unphysiological conditions in which their presence has been recognised, does have functional significance. It would be attractive to identify two of the stable states with the isologously paired heterotypic protein tetramers, postulated by Weintraub *et al.* (*Cell* **9**, 409; 1976), which are viewed as opening up to allow genetic readout, and with the electron microscopically-demonstrated splitting of the nucleosomes into two half-nucleosomes, described by Tsanev and Petrov (*J. Microsc. Biol. Cell* **27**, 11; 1976) and by Oudet *et al.* (*Cold*

Spring Harbor Symp. quant. Biol., **42**, in the press). Also relevant is a nice demonstration by Chung *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **75**, 1680; 1978), that a heterologous tetramer of histones can exist in solution in equilibrium with the octamer. At this stage such results as those of Gordon *et al.* must be seen as no more than a model to point the way for future studies, which will have to be performed also on polynucleosomes, both with and without their complement of H1 and nonhistone proteins. □

X-ray active galaxies and QSOs

from B. A. Cooke

Cosmic X-ray sources populate the sky in two spatial distributions. Some cluster towards the galactic equator and in resembling the distribution of visible stars seem to be within our Galaxy. The remainder, spread uniformly over the sky and usually of low intensity, are probably mostly extragalactic. Using the rotation modulation collimators of the SAS-3 satellite, K.M.V. Apparao and his colleagues recently attempted to measure more precisely the position of an X-ray source from the 4th Uhuru catalogue—4U0241+61 (*Nature* **273**, 450; 1978). The error box of 4U0241+61 (area 0.4 square degrees) overlapped with that of a γ -ray source from the COS-B Survey, CG 135+1 (3 square degrees) so that there was possibly an important connection between the two sources. The CG source is so close to the galactic plane (the +1 indicates 1 degree of galactic latitude) that, like the other CG sources, it is almost certainly of galactic origin.

The observations positioned the X-ray source within an error box only 30 seconds of arc across, which, after reference to sky survey plates, was found to contain a bright, extragalactic, stellar-like object having intense emission lines and a redshift of 0.0438. Apparao *et al.* had not located the γ -ray source but instead have added an important fourth member to the list of X-ray QSOs. The fact that some people might disagree with the description QSO, preferring to call it instead an X-ray Seyfert galaxy, reflects the present uncertainty of nomenclature in the field of active galaxies, whether they are X-ray active or not.

When QSOs were discovered, by the coincidence of bright, blue, stellar-like

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objects with radio sources. Their extraordinary luminosity compared with other astronomical objects gave them a uniquely elevated status. In fact controversy over whether their measured redshifts could be cosmological or not (allowing them to be nearer) is still not completely over. However, spectrally and in terms of optical variability, QSOs much resemble the (much nearer) type 1 Seyfert Galaxies in which an unusually bright (and thus active) galactic nucleus is surrounded by some sort of spiral or irregular galaxy. Furthermore, type 1 Seyferts share with QSOs considerably broadened hydrogen lines (frequently of full width $10,000 \text{ km s}^{-1}$) on top of a non-thermal continuous spectrum flat enough to produce an ultraviolet excess. At typical QSO redshifts this galaxy structure would be invisible and only a QSO-like, bright nucleus would be seen on an astronomical plate. Clearly at intermediate distances definitions designed to separate Seyfert galaxies and QSOs will conflict causing uncertainty in identification.

Seyfert galaxies have a luminosity range of about 1,000 but another factor of about only 10 in luminosity would be required in any model of this type of active galaxy in order to assimilate QSOs as well.

The current interest in the X-ray emission of active galaxies has come about in the following way. By making a sky survey more completely and more sensitively than had been possible before, the sky survey instrument on the Ariel V satellite revealed that the brighter type 1 Seyfert galaxies were invariably X-ray emitters. The object discovered by Apparao *et al.* is heavily reddened by obscuration in the galactic plane but correction for this leads to an absolute optical magnitude about 3 magnitudes brighter than the most luminous type 1 Seyfert! Had the object been well out of the galactic plane it would have already been seen during the Ariel V survey and probably classified as an unusually luminous Seyfert galaxy. It is also valid to call it a QSO, however, since its luminosity is close to the average for QSOs and there are some QSOs at a similar redshift. As yet none of the X-ray active galaxies has surpassed the first discovered X-ray QSO, 3C273 which, both optically and in the X-ray region, is a very luminous QSO by any standards.

This latest X-ray discovery adds valuable strength to a bridge being built (alongside one on the optical regime) connecting nearby active galaxies with QSOs. The Ariel V and SAS-3 satellites are only just sensitive enough to be able to probe the region where Seyfert galaxies and QSOs 'co-exist'. There can be no doubt that when results from the more sensitive

survey by the HEAO-A satellite become available the list of new X-ray sources will be found to contain many new X-ray Seyferts and QSOs. The deep X-ray 'plates' and studies of individual X-ray objects by HEAO-B should further enhance our appreciation of the connection between them. We look forward to a wealth of observational material and anticipate a new understanding of the mechanisms which keep these objects so outstandingly luminous. □

Size and symmetry

from a Correspondent

THE experimental investigations of the atomic properties of matter have been generally confined to matter in the gaseous state (single atoms and molecules), the solid state and, only more recently, to the nature of liquids. The structure and formation of matter in a state intermediate between that of the gas and liquid phases, that is, the polyatomic cluster, has only recently been subjected to experimental investigation. The exact nature of the bonding in these clusters is at present unknown. Such cluster grouping which forms a bound stable atomic system must be distinguished from the formation of a polyatomic molecule since the forces holding the cluster together might not be predominantly chemical in origin but possibly derived from Van der Waals interactions.

Since alkali metal atoms are easily detected by surface ionisation and can be deflected in non-homogeneous magnetic fields they constitute a very useful group of elements for atomic beam experiments. Cluster formations of alkali atoms can be produced by simply evaporating the alkali metal in an inert argon atmosphere at 10^{-3} torr (Kimoto & Hishida *J. Phys. Soc. Jap.* **42**, 2071; 1977) or by nucleation of an alkali vapour beam in a high pressure, 800 torr, argon atmosphere followed by expansion of the gas through a supersonic nozzle (Knight *et al. Phys. Rev. Lett.* **40**, 1324; 1978). Clusters containing up to 100 atoms have been produced in this way.

Alkali atoms are known to form weakly bound dimers (for example, K_2 dissociation energy is 0.51 eV) and these diatomic systems have been studied spectroscopically in the gas phase (Demtröder *et al. J. Chem. Phys.* **51**, 5495; 1969) and are well understood. In addition, trimers of sodium have been produced by condensation of Na_3 in an argon matrix on a liquid helium cooled sapphire surface. Elec-

tron spin resonance spectroscopy shows that in Na_3 (Lindsay *et al. Molec. Phys.* **32**, 1199; 1976) the unpaired electron is in an s orbital, not a p orbital, and that 90% of the spin density is distributed between two atoms indicating that trimers have predominantly chemical bonding and not just Van der Waals forces binding the atoms together. Even with atom-dimer beam scattering experiments (Whitehead *Molec. Phys.* **31**, 549; 1970) and spin polarisation studies (Kempitsas & Weber *Chem. Phys. Lett.* **35**, 277; 1975) it is still not possible to differentiate completely between Van der Waals and chemical bonding in even small clusters.

An understanding of the nature of the bonding in clusters, as a function of the cluster size, might also provide some further insight into the structure of liquids. To date only a very limited amount of experimental data is available on clusters although the method of cluster production has been known for many years. The photoionisation potentials of sodium clusters (Foster *et al. J. Phys. B.* **2**, 478; 1969) show a progressive decrease in the binding energy with cluster number. Measured ionisation potentials are 5.41 eV for a single atom falling to 4.2 eV for a four atom cluster indicating a trend towards the work function (2.3 eV) of the metal in bulk.

Kimoto was unable to produce clusters of 3 or 5 lithium atoms and only very small fractions of clusters of 7 and 9 lithium atoms although the even clusters of 2, 4, 6 and 8 atoms were produced in large quantities. Since no spectroscopic data is available the structure and bonding in these multiple atomic systems is a matter for conjecture. Although the nature of bonding is unknown the existence of odd-numbered clusters of potassium atoms has been demonstrated by Knight *et al.* In this case detection of the clusters was by deflection in an inhomogeneous Stern-Gerlach magnetic field. Only odd-numbered clusters were detected since even combinations of potassium atoms always combine with net zero spin and hence zero magnetic moment whereas odd-numbered clusters will have one unpaired electron and hence a magnetic moment of $1 \mu_B$. Hence a pentamer will experience a deflection of $1/5$ that of a monomer in the same magnetic field. With an improved apparatus Knight *et al.* hope to resolve spatially clusters of 21 atoms.

Since adequate sources of clusters are now in existence, the investigation of the collective atomic properties of these systems using the techniques of molecular beam resonance, microwave spectroscopy

and photon interaction is now possible and desirable.

Hence we see that when atoms group together to form a complex the atomic properties of the constituent atoms are altered. The modification of the properties of isolated atoms is not only evident in clusters, liquids and solids but also in the different types of chemically bonded polyatomic molecules. The Auger electron yield is a property characteristic of a particular atom but it has been reported (Mathews & Hopkins *Phys. Rev. Lett.* **40**, 1326; 1978) to be dependent on the symmetry and size of the molecule containing the atom. Matthews and Hopkins have investigated the Auger electron LMM yield for sulphur atoms in H_2S , SO_2 and SF_6 and the KLL electron emission from carbon in CH_4 , CCl_4 and CF_4 . They have reported that the carbon KLL Auger cross section is largest for CH_4 , falling by 11% for CF_4 and 25% for CCl_4 . Although all of these molecules are tetrahedral the lowest yields are obtained from the molecules which have the largest electron density in the

region surrounding the central carbon atoms. In the case of the sulphur LMM electron yield the variation is much more apparent. The cross sections for the characteristic LMM yield in the linear H_2S and SO_2 molecules are the same but this yield is reduced by 50% in the octahedral SF_6 molecule. In addition an anomalous Auger electron yield is detected at 50 eV below the characteristic peak position. Nevertheless the sum of the anomalous and reduced characteristic yields equals the total expected yield.

Matthews and Hopkins argue that the changes in the Auger yield are not a result of a variation in the binding energies of the sulphur 2p or carbon 1s electrons but due to the influence of the valence electrons on the Auger electrons as they travel out from the centre of the molecule. They suggest that the Auger yield is modified by energy loss collisions or possibly other mechanisms associated with the valence electron cloud, for example, potential-barrier scattering or a double Auger process. □

is not an inevitable conclusion. Evidence of this sort has recently been presented by Pearsall (*Science* **199**, 177; 1978) for soils derived from the archaeological sites of Real Alto, Ecuador. The presence of large phytoliths in these samples led her to examine the possibility of defining maize phytoliths on the basis of size criteria and thus determining whether maize was grown in the area in the third millennium BC.

Pearsall examined the native grass genera having the same, cross-shaped phytoliths as maize and found that their silica bodies all fell below $16\text{ }\mu\text{m}$. Various modern races of maize were also examined and they produced phytoliths ranging from 6 to $25\text{ }\mu\text{m}$, but overall 32% of their phytoliths exceeded $16\text{ }\mu\text{m}$. In the archaeological material, 100 phytoliths were counted in each of 17 samples. Twelve samples contained the cross-shaped type, a total of 42 in all. Of these 15 (36%) exceeded $16\text{ }\mu\text{m}$ in size. Pearsall interprets this as indicative of maize cultivation on site. What is needed now are more extensive numerical analyses of modern phytoliths to test the reliability of size criteria and also further work on the movement of phytoliths in soils, plant debris and dust to establish their potential for long-distance transport. These microfossils could be a valuable source of information for palaeoecologists which is currently underexploited. □

Botanical fingerprints

from Peter D. Moore

DEPOSITS of silica in the epidermal cells of grasses produce distinctive 'silica bodies' or phytoliths, which can take on a considerable variety of forms. The shape of phytoliths is reasonably constant within a species, but size varies. Distinctions between species on the basis of shape has not proved very successful and often it has not been possible to proceed beyond the tribe level in phytolith identification.

The desire to identify phytoliths has arisen largely because of palaeoecological studies on both soils and peat deposits. Since these resistant, siliceous structures survive when the bulk of the grass litter decays, they remain as microfossils in a variety of situations. For example, Finney and Farnham (*Proc. 3rd Int. Peat Congr., Quebec*, 102; 1968) recovered phytoliths from the inorganic fraction of peat deposits from northern Minnesota. This involved a slow oxidation of peats for up to three weeks using H_2O_2 . They found high concentrations of phytoliths in the superficial layers of peat (over 5% of oven dry weight) at the two sites which they examined, and this can probably be associated with human management

of grassland and consequent soil erosion by wind in recent times. In other aspects the two sites differed. One, at which peat accumulation commenced soon after deglaciation, showed initial low phytolith abundance which increased gradually about half way up the 5 m profile. At the other site, phytolith abundance rose in the basal peats and remained high; this site was probably younger, perhaps 6,000–7,000 years old. Finney and Farnham account for the increasing abundance of phytoliths within the post-glacial in terms of contemporaneous expansion of prairie vegetation in the west and south of Minnesota.

Phytoliths can thus provide a useful index of grassland history and of human land use patterns. Also, their abundance in ombrotrophic peats which receive water and nutrient input from rainfall rather than from drainage water, suggests that phytoliths are carried from place to place as airborne particles.

Phytolith microfossils survive in soils (see, for example, Jones & Beavers *Soil Soc. Amer. Proc.* **27**, 438; 1963), where they can be interpreted as providing evidence of grass species growing *in situ*, although given the possibility of aerial transport as dust, this

Erratum

In the article 'gamma-ray spectroscopy in astrophysics' (*News and Views* **273**, 591; 1978) the second author's name should be T. L. Cline and not T. L. Kline.



A hundred years ago

A Subject-Index to Scientific Periodical Literature

I BEG permission to ventilate in your columns a subject which must make itself felt more or less to all your readers, viz., the want of some subject-index to the vast amount of material scattered about in the numerous scientific periodical publications of the present day. It is true we have the admirable catalogue of the Royal Society, but unless you are acquainted with the name of every author who has written on your subject, it is nearly hopeless attempting a complete bibliography of it.

From *Nature* **18**, 4 July, 251; 1878.

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Does carcinogenic potency correlate with mutagenic potency in the Ames assay?

Bruce Ames and Kim Hooper reply to a recent criticism of their inquiry into the relation between mutagenic potency in the Ames test and carcinogenic potency.

IN a recent article in *Nature*¹ Ashby and Styles of ICI criticise the approach of the pioneering paper by Meselson and Russell² and of our unpublished work, both of which explore the relation between the potency of a chemical in an animal cancer test and its potency in the *Salmonella* mutagenicity assay³. Their criticisms suggest first that an "invariable correlation" has been proposed, which is not true, and second, that it has been accepted by part of the scientific community, which is unlikely. Meselson's study was only exploratory, and his analysis of 14 chemicals showed a good, but not perfect, correlation. Our much more extensive study is still in progress, and we would have preferred for Ashby and Styles to have waited until it was completed and then evaluate it on its merits. We find it unusual to have to reply to an attack that distorts our unpublished work. We think our analysis of the potency of carcinogens and of mutagens is necessary to evaluate the strengths and quantitative limitations of both animal cancer tests and short-term tests. Carcinogenesis is clearly complicated, which is all the more reason to ask critical questions. Unfortunately, most of the examples that Ashby and Styles present as counter-evidence to a correlation are poorly thought out or erroneous in some essential aspect, and serve only to confuse⁴ rather than clarify the issues. They have also ignored Meselson's published data. We will briefly discuss only the more important points.

The major aims of our current study are

- (1) To analyse the published literature on animal cancer tests so as to develop methods for calculating carcinogenic potency. We (C. Sawyer, K. H. A. Friedman and B. N. A. R. Peto) have extended a theoretical analysis² and also have analysed more than a thousand papers to calculate for a large number of carcinogens the daily dose of a chemical that will give half of the experimental animals cancer in a lifetime feeding experiment. This dose (the TD₅₀) can vary by more than a million-fold, which makes its determination both important and particularly relevant to human risk assessment.
- (2) To develop a measure of the thoroughness of a negative cancer test. This is expressed as a 'no greater than' potency value for a particular test. Given the enormous variation in thoroughness of animal cancer tests, it is essential to be able to state the sensitivity of any test in which a compound is found to be a 'non-carcinogen.'
- (3) To establish a set of reference points of carcinogenic potency for calibration of short-term tests. Carcinogenic potency values for the rat can be compared with mutagenic potency values in the *Salmonella* assay using liver homogenate from rats; both show a million-fold range^{2,5-7}. This calibration of short-term tests will identify the chemicals for which each test needs improvement and for which there is a correlation with *in vivo* potency.
- (4) To determine the extent of species (or sex or strain) variation in carcinogenic potency and to what extent a difference is attributable to metabolic activation. This can be tested using liver (or other tissue) homogenate from the different species to measure activation of the chemical to a mutagen in the *Salmonella* test. Insofar as species

differences seem to be explainable by metabolic activation, we can determine the activity of autopsy human liver (or other tissues if necessary) in our test. Ashby and Styles dwell on species variation as a key point, implying that it is naive to look at potency because of it. They misunderstand what we are trying to do. It is an advantage, not a disadvantage, of our test that we can use the tissue homogenates from different species.

- (5) To see to what extent potency values from a battery of short-term tests can be used as an aid in human risk assessment and for setting priorities in dealing with the very large number of environmental carcinogens and mutagens. There is a critical need for identifying mutagens and carcinogens among the 40,000 man-made chemicals in commercial use to which humans are exposed (with a thousand more being introduced each year) and among the many natural carcinogens present in complex mixtures⁸. We must identify carcinogens and mutagens and set priorities for dealing with them on the basis of their potency and the degree of human exposure to them. Human epidemiology and animal cancer tests alone are completely inadequate for this enormous task. A battery of short-term tests is a promising new tool which may provide us with the information we need for getting a rough idea of potency in order to set priorities. The alternative of using people as guinea pigs is no longer acceptable.

Given the million-fold range in carcinogenic and mutagenic potencies, a correspondence between them can be useful without needing to be very precise. That there is a good correlation for most chemicals examined so far is suggested by the work of Meselson and Russell², by our own work to date, and by our comparison of a number of carcinogens with their less active isomers^{5-7,9}. In addition, it seems that some of the other short-term tests show potency responses similar to that of *Salmonella*. Potency values in a mammalian short-term test (inhibition of DNA synthesis in human HeLa cells) have recently been obtained for 16 chemicals¹⁰ and show a million-fold range, as well as a remarkably good agreement with our *Salmonella* potency results.

The theoretical basis for the correlation we do see may be that using rat liver homogenate as a model for a rat's metabolism of foreign chemicals is a reasonable first approximation, especially because we are only analysing the carcinogenic potency for chemicals that are ingested. The liver is the primary organ for metabolising foreign chemicals and in general is much more active than other tissues for metabolic activation. (Most carcinogens that are ingested do not cause liver cancer, but this may be explainable by the increased DNA-repair capabilities of the liver¹¹.) Our analysis should, in any case, give some indication of the chemicals for which it is necessary to use tissues other than liver in mutagenicity tests.

Ashby and Styles indicate they do not find a million-fold range in potency in the *Salmonella* test. There is, in fact, more than a million-fold range in potency in *Salmonella*⁵⁻⁷, as shown in many laboratories, and this is the heart of our disagreement. They may not be able to repeat our results for the following reasons. They have considerably lowered the amount of co-factors in the assay^{12,13}, which we know can markedly lower the

mutagenic response. Their culture of *Salmonella typhimurium* TA100 has mostly lost the R factor as shown by their very low spontaneous mutation rate for this strain^{12,13}. The inadequacy of their experiments can be seen quite dramatically by comparing the experiment in their Fig. 3 in ref. 1 using benzo(a)pyrene (B(a)P) and PCB-induced rat liver homogenate, with our published experiment³. Their Fig. 3 shows fewer than 100 revertant colonies (for 500 µg of B(a)P with PCB-induced rat liver) and no linear dose response. We report over 400 colonies with only one-hundredth of this amount of B(a)P (5 µg) using the same strain, TA1538 (ref. 3). With our more sensitive strain TA100, we report in the same paper a linear dose response from 1 to 5 µg of B(a)P with 2,000 colonies at 5 µg. We find linear dose responses for almost all of the hundreds of mutagens we have tested^{5,6} and have published a large number of these. Ashby and Styles also seem to misinterpret potency by not determining it from the linear part of the dose-response curve^{12,13}.

The conceptual and technical errors in their experiments (Figs 3, 4, and 5 in ref. 1) make it difficult to draw any conclusions from them. In our methods paper³ we warn against the error of comparing two enzyme (liver homogenate) preparations at only one enzyme concentration without first showing that adding more enzyme increases the activity (number of revertant colonies) proportionally. In our experiment with B(a)P, using a series of increasing enzyme concentrations, the activity increased and then dropped when more than 50 µl of enzyme was added per plate (the maximum we recommend)³. Thus, Ashby and Styles's experiments may be performed on a descending part of a curve so that the less active liver preparation seems more active. This may account for their error in concluding that PCBs do not induce B(a)P activation activity in rodents—the opposite conclusion to that of many papers in the field and of our own work³. The experiments comparing liver homogenates from other rodents with that of the cotton rat, which Ashby and Styles quote and accept quite uncritically, is another example of this same methodological error. The cotton rat experiment needs to be done, but it has not been done correctly.

We do not disagree with the general point Ashby and Styles are trying to make, that since we have developed a standard set of test conditions to detect most carcinogens with maximum sensitivity, varying the parameters in the test could change potency somewhat. This is one of the many-reasons why this kind of correlation is never going to be very precise or likely to hold for every class of chemical. Ashby and Styles, however, cite counter examples in which potencies are compared over too small a range, or in which the measure of the dose (necessary to determine carcinogenic potency) is not clear (for example, the polycyclic skin painting study).

Ashby and Styles emphasise the problem of the false negatives and false positives in the *Salmonella* test. The test does miss a few important classes of carcinogens⁵⁻⁷. Some of these will never be detected because they cause cancer by non-mutagenic mechanisms, or for other reasons which we have discussed⁵⁻⁷. Others may be detected after further improvement of the test⁷. None of the short-term tests is perfect (*Salmonella* detected about 90% of the carcinogens examined)^{5-7,12-14}. Animal cancer tests have their own inherent problems, and one must look just as critically at the evidence for 'non-carcinogenicity.' We have discussed why we think the few false positives in the *Salmonella* test^{5-7,9} may be carcinogens if given more thorough animal cancer tests. One striking illustration is the Japanese food additive AF-2. It was consumed in enormous amounts for close to a decade after being found negative in two animal cancer tests. It then was found to be mutagenic in a variety of short-term tests, including *Salmonella*, and after more thorough retesting has been found positive in several animal cancer tests (see several papers in ref. 8). Many short-term tests, including *Salmonella*, have successfully predicted carcinogenicity for a number of important environmental chemicals with widespread human exposure,

including the flame retardant *tris*, the major industrial chemical 1,2-dichloroethane (ethylene dichloride), and a series of hair dye chemicals. We expect that a battery of short-term tests⁸ will soon be quite useful in estimating carcinogenic potency as well.

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Factors influencing mutagenic potency *in vitro*

JOHN ASHBY AND J. A. STYLES REPLY: We would reiterate that the *Salmonella* assay is invaluable for detecting potential animal carcinogens, especially if used in conjunction with a second *in vitro* assay. However, attempts to compare carcinogenic potency with mutagenic potency in this assay should acknowledge the established variability of *in vitro* mutagenic effects. It has been demonstrated that the observed mutagenic

- (1) Choice of tester strain (at least 24 are available of which about 8 are regularly used)^{12,25}.
- (2) Choice of test protocol: plate incorporation¹², liquid culture²⁶, fluctuation²⁷ or host-mediated²⁸.
- (3) Choice of organ from which the S-9 mix is prepared^{29,30} (usually liver).
- (4) Choice of species from which the liver S-9 mix is prepared^{13,31,32}.
- (5) Choice of liver enzyme-inducing agent: Aroclor 1254 (ref. 12), 3-methylcholanthrene¹², phenobarbitone¹², folate deficient diet³³ (and so on), or non-induction^{4,12}.
- (6) The volume of S-9 mix used and the balance therein between S-9 fraction and co-factors^{12,13}.
- (7) Addition of purified enzymes to the S-9 mix^{16,17,34}, or depletion of enzymes through sub-fractionation of the initially derived S-9 fraction^{13,34}.
- (8) Addition of non-mutagenic adjuncts, such as norharman⁵⁻⁷ or nitrososarcosine³⁵, to the assay medium.
- (9) The time of incubation of the bacteria with the test chemical¹.
- (10) Choice of anaerobic or aerobic incubation conditions^{8,37}.
- (11) Choice of test-chemical solvent (D. Anderson, unpublished results).
- (12) The salt²⁵, buffer^{36,37} and nucleophile content³⁸ of the assay medium.
- (13) Definition of countable colonies and the method of colony counting chosen (E. Longstaff, unpublished results).
- (14) As yet undefined variables, which when documented might account for why carcinogens such as dimethylnitrosamine^{14,26}, safrole^{14,39,40} and hexamethylphosphoramide²³ give a changed response (+ → - or - → +) on retesting under apparently identical conditions. Such undefined factors might also explain why some compounds produce an apparent positive effect in the assay without mutating the bacteria. (This may be due to phenotypic reversion. Two compounds possessing this property have been identified, E. Longstaff, unpublished results.)

Cotton rat anomaly

D. B. MCGREGOR REPLIES: Some years ago experiments performed at this laboratory¹ indicated that 2-acetylaminofluorene (AAF) could be more efficiently activated to a mutagen detectable by *Salmonella typhimurium* TA1538 if the postmitochondrial supernatant (S-9) was derived from cotton rat liver than if the S-9 came from rat liver. Carcinogenicity tests demonstrated, however, that 2-AAF may be non-carcinogenic for cotton rat whereas the compound is known to be a powerful carcinogen for rats². Metabolite binding to DNA in various species of rodents correlated much better quantitatively with the carcinogenicity tests than did the *in vitro* mutagenicity assays (Ames' test).

While there is nothing in these results to refute the qualitative correlation between mutagenesis and carcinogenesis, there seems to be a caution against quantitative extrapolations. This argument may, however, be at fault because of the design of the mutagenesis tests: relatively high levels of S-9 were used (150 μ l per plate) and studies by several workers (ref. 3 for example) have shown that there is often an optimum S-9 level which can produce maximal response. This optimum could occur at different doses of S-9 derived from different sources. For studies on reaction kinetics, S-9 concentrations below this optimum should, arguably, be used, although the value of such studies in the context of the Ames test as a quantitative predictor of carcinogenesis is minimal. In any carcinogenesis test, or inadvertent human exposure to a potential carcinogen, there is almost always a massive excess of metabolic potential compared with the dose of carcinogen. Therefore, the importance of using small quantities of liver S-9 in a mutagenesis test is questionable. Nevertheless, the experiment with smaller quantities of S-9 has been performed and demonstrates at all S-9 concentration levels that cotton rat liver is more efficient in 2-AAF activation than is normal rat liver (Fig. 1). Induced liver enzymes may well give different results, but the importance to quantitative carcinogenesis correlations of mutation frequencies obtained in the presence of induced liver S-9 must be less than results with normal liver S-9. Such correlative exercises have recently become an interesting aspect of genetic toxicology, but they are in an immature state of development.

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potency of a chemical in *Salmonella typhimurium* is influenced by at least fourteen major factors (see box). It is therefore not surprising that during the past 3 years the response given by the Ames assay to several compounds has changed from reproducibly negative to reproducibly positive following modifications made to the test protocol. Examples are the fluorocarbon F22¹, the fire-retardant Fyrol FR2 (ref. 2), ascorbic acid³ butter yellow⁴⁻⁶, aniline⁷, *o*-toluidine⁷, azathioprine⁸ and styrene⁹⁻¹¹. Further, the comparison by Ames and Hooper between the two sets of results for benz(a)pyrene^{12,13} indicate that changes made to the S-9 mix (point 6 in box) can give rise to at least a 4×10^2 -fold variation in the observed mutagenic response of a chemical routinely detected by this assay.

The procedural modifications listed below and their effect on the mutagenic potency of a chemical are acceptable if this assay is to be used for its original purpose, namely, to detect potential carcinogens^{12,14}. This was the single objective of the study of Purchase *et al.*¹⁴. If, however, an attempt is to be made to

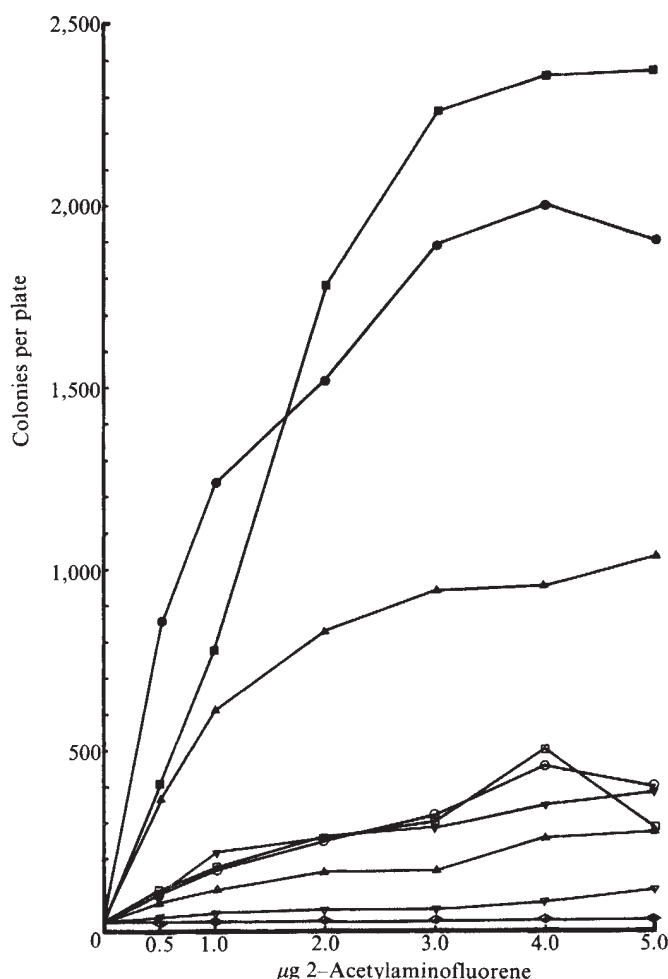


Fig. 1 Activation of 2-acetylaminofluorene to a mutagen in the Ames test by postmitochondrial supernatant preparations (S-9 mix) from either male Wistar rats or male cotton rats. The detector organism was *Salmonella typhimurium* TA1538. The data points are mean counts from six plates derived from two experiments. Each S-9 mix consisted of 4 μ M NADP, 5 μ M glucose-6-phosphate, 8 μ M $MgCl_2$ and 33 μ M KCl in 0.05 M phosphate buffer, pH 7.4. The volume of S-9 in each 500 μ l S-9 mix varied as follows: 10 μ l (rat and cotton rat $\diamond$); 25 μ l (rat ∇ , cotton rat $\blacktriangledown$); 50 μ l (rat $\triangle$, cotton rat $\blacktriangle$); 100 μ l (rat $\circ$, cotton rat $\bullet$); 150 μ l (rat $\square$, cotton rat $\blacksquare$).

correlate mutagenic with carcinogenic potency, a fixed *in vitro* assay protocol must be adopted, but the suggestion made in the above letter, that the 1975 methods paper of Ames *et al.*¹² defines this protocol, seems premature. This is mainly because the majority of the variables (see box) were defined after 1975, and many are significant as they enabled individual carcinogens or potential carcinogens to be detected by this assay for the first time. Similarly, the criticism that the methodology adopted in our study¹³, or that of Purchase *et al.*¹⁴ or that of McGregor¹⁵ (cotton rat experiment) is in error is unfounded since a test protocol that takes account of points 1-14 has yet to be defined.

It is also to be expected, given this list of variables, that some chemicals will produce mutation *in vitro* through formation of metabolites not associated with those which cause cancer *in vivo*, specific examples being benz(a)pyrene^{16-18,41} and 7,12-dimethylbenz(a)anthracene¹⁹. In such cases it is difficult to envisage any relationship between these unconnected responses²⁰.

The basic assumption suggested in the paper by Meselson and Russell²¹ that "the carcinogenic potency of a mutagenic compound for man may be roughly equal to that in rodents" clearly focuses the need to decide which species of rodent and which test protocol most closely resemble the manner of xenobiotic transformation of chemicals in man. Further, it is probable that the large majority of mutagens will give an *in vitro* response within a 10³-fold range (Meselson and Russell²¹ observed this range for 90% of their mutagenic carcinogens) and that the limits of this range will be populated by a few very potent or very weak mutagens. It is also important to remember that if a procedural modification is found which enables, for example, the very potent carcinogen hexamethylphosphoramide²² to be detected as a very potent mutagen (it is usually negative²³) it is probable that general use of such a protocol would dramatically affect the mutagenic response given by previously evaluated chemicals. The absence of a requirement to determine cell-survival data will make such reviews difficult.

Correlations between mutagenic and carcinogenic potency may be found in some specific cases, and these may prove useful as a research tool, but they could easily assume the mantle of an invariable correlation. This would be wrong²⁴ and would not assist the task at hand.

Others are involved with *in vitro* testing within our laboratory, and more than one paper has been discussed above. This reply carries the support of our whole group.

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review article

The context of the search for axions

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Should axions exist, their discovery would be a major scientific advance. The facts and the motivation underlying the recent search for these conjectured new elementary particles are outlined in this review.

AXIONS, new elementary particles of mass less than or comparable to electrons, might have escaped previous detection because they would be semi-weakly interacting, neutral and pseudoscalar, and hence somewhat elusive. Their discovery would have been hailed as a simultaneous triumph of modern experimentation and the whole philosophic thrust of contemporary gauge field theories.

Background

As soon as the axion (or higglet), a^0 , with its rather specific and unusual properties was proposed^{1,2}, careful thought was given as to how and where it should be seen. Several electromagnetic

and weak decay situations were examined, but in all cases the predicted effects turned out to be too small to establish with existing techniques¹. A better prospect seemed to be offered by reactor neutrino experiments in which an enormous flux of a^0 s should be produced, entailing an appreciable count rate from secondary photons (for example, from a^0 to $\gamma\gamma$) in the NaI detectors, and of neutrons from disintegration of deuterons ($a^0 + d \rightarrow p + n$) in the D₂O moderator. Reported experimental limits on these signals lie many orders of magnitude below the expected rate.¹ Thus, the first potentially sensitive test of the axion hypothesis gives a negative result. However, the couplings of the axions could be such that they 'tune out' the reactor signal; in any case, it would be nice to confirm the result in another context, ideally in some simple situation in which axions and their reactions would predominate.

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The upsurge of theoretical activity which led to the axion idea fortunately coincided with the running of a massive 'beam dump' experiment at CERN³⁻⁵ (for a general discussion see refs 6, 7). An intense 400 GeV proton beam is 'dumped' into a 13 interaction length thick copper target upstream of a very large composite neutrino experiment comprising an enormous thickness of shielding ($\sim 1,000$ s of interaction lengths) followed by three very elaborate and highly instrumented neutrino detectors in series: (1) the large hydrogen bubble chamber BEBC filled on this occasion with a 70% neon-hydrogen mixture³, (2) the very large CDHS neutrino counter detector⁴, and (3) the heavy-liquid bubble chamber Gargamelle filled with heavy freon, CF₃Br, and with a 'useful' interaction volume of 7 m³ (ref. 5). Only weakly or semi-weakly interacting neutral particles (that is, various species of neutrinos (ν s) with interaction proportional to G_w , or one or other of the conjectured gauge field quanta with interaction proportional to $G_w^{1/2}$, either neutral intermediate vector bosons (Z^0) or the associated scalar Higgs particles (h)) can get through the massive shield; the thick target prevents ordinary π and K decay from appreciably feeding the weak flux. The prime outcome has been an important positive discovery^{3-5,7}—the observation of an unexpectedly large flux of 'prompt' neutrinos, which probably arises from the decay of charmed particles. However, the same setup affords a very sensitive detector for axions.

Hypothetical properties

The crucial parameter determining the hypothesised properties of axions is the dimensionless ratio $\xi \equiv f_\pi/f_w \approx 2 \times 10^{-4}$, where $f_\pi \approx 180$ MeV is the pion decay constant, and $f_w \equiv 2^{-1/4} G_w^{-1/2} \approx 250$ GeV measures the inverse strength of weak interactions⁸. In the models in which axions arise⁹, the axion mass, m_a , is of the order $\xi m_\pi \sim 100$ keV, and its lifetime, τ_a , associated with the decay $a^0 \rightarrow \gamma\gamma$, of the order $(\text{const}) \times \tau_\pi/\xi^5 \sim 10^{-2}-10^{-1}$ s (refs 1, 2). Its principal interaction with ordinary matter (production and absorption) arises from 'mixing' with its low-mass strongly interacting counterparts, π^0 and η^0 , this mixing also being of the order ξ . In the beam dump experiment, the a^0 s should be made in the front layers of the target, proceed through all the intermediate layers of material with negligible attrition from decay or absorption and pass into the detectors. There, they should show up by the unmistakable signal of anomalous neutral-current type events ($a^0 + A \rightarrow \text{hadronic fragments (+ no charged lepton)}$) however, distinguished from ordinary neutral current events ($\nu + A \rightarrow \nu' + \text{hadronic fragments}$) by the absence of unbalanced transverse momentum. The observed rate should be proportional to the product of the production cross-section (σ_p) in the target and the absorption cross-section (σ_i) in the detector. The resultant parameter ($\sigma_p^a \sigma_i^a$) can be related to the corresponding π^0 coefficient through the parameter ξ to yield an absolute estimate for the rate to be expected. In particular, Ellis and Gaillard¹⁰ using Weinberg's current algebra model for the interaction of axions¹, have estimated an absolute lower limit: $\sigma_p^a \sigma_i^a \geq \frac{32}{9} \xi^4 (\sigma_p^{\pi^0} \sigma_i^{\pi^0}) \approx 10^{-65} \text{ cm}^4$, for this quantity, independent of the relative mixing of a^0 with π^0 and η^0 . It is remarkable that this tiny predicted limit is excluded by the above three experiments, which find for the ratio $R \equiv (\sigma_p^a \sigma_i^a / \sigma_p^{\pi^0} \sigma_i^{\pi^0})^{1/2}$, which theoretically¹⁰ should be $\geq (\sqrt{32/3}) \xi^2 \approx 8 \cdot 10^{-8}$, the limits: $R < 0.7 \times 10^{-8}$, $R < 0.5 \times 10^{-8}$. ($a^0 A \not\rightarrow X$ and $a^0 \text{Fe} \not\rightarrow \mu^+ \mu^-$); $R < 10^{-8}$ (refs 3-5).

Thus, a negative result from the high-energy neutrino experiments reinforces the reactor neutrino measurements. Whether it proves decisive remains to be seen—the experiments are new and have problems of mutual consistency in other aspects of their results; the theoretical estimates could obviously be evaded by complicating the model. (Various well motivated extensions are discussed in ref. 27.) Whatever the outcome, it is interesting to examine the genesis of the axion idea as an illustration of the methods of contemporary gauge theorists and of the highly speculative and yet extraordinarily

closeknit and philosophically satisfying web of concepts which they have developed. (Ref. 1 gives an extensive list of references to the antecedent theory. For general reviews see refs 8, 11.)

Discussion

Axions arise in classes of models⁹ which generalise the usual Higgs mechanism whereby the intermediate vector bosons acquire mass through coupling to a complex scalar field with non-vanishing vacuum expectation value. They are pseudo-scalar Higgs particles, semi-weakly coupled to quarks and leptons; in the simple variants of the standard $SU(2) \times U(1)$ Higgs couplings which have actually been considered, they are the Higgs particles of the model^{1,2,12}. Although, as already implied, their discovery would have been a triumphant vindication of a whole interconnecting network of theoretical ideas, their non-appearance is by no means a disaster. The object in complicating the Higgs couplings was to stabilise the theory against unwanted and uncontrolled parity and time-reversal (PT) violating effects feeding through from the strong (S) to the weak and electromagnetic (WEM) interactions. It was only recently perceived that the strong interactions could generate such effects this being connected with the unquestioned existence of 'instanton' effects (quantum coupling between topologically distinct gauge vacua) in 'non-Abelian' gauge theories and, in particular, in the conjectured gauge theory of the strong interactions, $SU(3)^{\text{colour}}$ (QCD). The Peccei-Quinn⁹ mechanism gets rid of this problem in a way which accords with the maxim that small parameters should arise 'naturally' (that is, without contrived tuning of input parameters) which gauge theorists cherish. (For a progress report on gauge theories of strong, weak and electromagnetic interactions see ref. 13; for a general review of quantum chromodynamics (QCD) see ref. 14.)

Theorists have been preoccupied with the interface between the (S) and (WEM) interactions ever since the discovery of the (V-A) structure of the weak lepton currents and its approximate replication by the weak hadron currents (V-1.2A). If one assumes an identical basic (WEM) interaction for leptons and hadrons (in modern parlance for quarks), how can one guarantee that the (S) interactions should not totally obscure the (WEM) symmetry? Answering this question has generated a long sequence of theoretical ideas: First, it is supposed that the underlying (S) interactions possess a chiral symmetry over and above the various 'internal' symmetries associated with flavour and colour. The idea is that the interactions of the elementary spin- $\frac{1}{2}$ fermions $\{f\} = \{\text{quarks; leptons}\}$ are such as to admit a zero order classification into left-handed and right-handed states $|f_{L,R}\rangle = \frac{1}{2}(1 \pm \gamma_5)|f\rangle$; however, the associated symmetry is broken by simple mass terms in the dynamics (note that only in the limit $v/c \rightarrow 1$ do the $|f_{L,R}\rangle$ states correspond to left (right) hand spin orientation). One is thus committed to an intrinsically 'broken' type of symmetry, a symmetry which, furthermore, is apparently broken in a specific and distinctive way, unanticipated in the original formulation of quantum field theory.

To characterise the symmetry breaking, one needs a formula for the divergence of the axial current, $D^\alpha \equiv \partial_\mu J_\mu^{\alpha A}$ (α denotes internal quantum numbers of the axial current, J_μ^α , for example, $\alpha = \pi$ for the π -like current). The form of D^α determines the non-conservation of the corresponding axial charges, $Q^{\alpha A} \equiv \int d^3x J_0^{\alpha A}$; these charges are the generators of the associated chiral transformations that distinguish left- and right-handed states. The D^α are evidently non-zero, for example, $D^\pi \neq 0$ because pions decay ($\pi^+ \rightarrow \mu^+ \nu$) (ref. 16). This observation is encapsulated in the PCAC hypothesis, $D_M^\alpha = ((m_\alpha)^2/f_\alpha)\phi^\alpha$, which relates the axial divergences to associated pseudoscalar fields, ϕ^α . In its strong form, PCAC entails assuming that the symmetry limit of the corresponding chiral algebra is realised spontaneously, in the Nambu-Goldstone fashion (the vacuum is regarded as a non-unique but degenerate configuration such as commonly occurs in the

ground state of a many-body system); the pseudoscalars are the 'Goldstone bosons' associated with the degeneracy¹⁵. If the symmetry were exact, these would be massless; through symmetry breaking, usually thought of as arising from small quark mass terms in the basic interaction, they acquire mass. From this viewpoint, the PCAC relation reads as an expression for the pseudoscalar mass, m_π , in terms of the coupling of the pseudoscalar field, f_π , times the intrinsically strong-interaction quantity, $\langle D^a \rangle$. This is the basis of the estimate for the axion mass, $(m_a)^2/(m_\pi)^2 \approx f_\pi/f_a$, referred to above, and also underlay a formerly emphasised difficulty with the η mass, the so-called 'U(1) problem'¹⁷ (it was thought that D^0 ought to resemble D^0 so as to predict $m_\eta < \sqrt{3}m_\pi$).

The above contribution to D^a was labelled D_M^a ('M' to suggest quark mass effects) to distinguish it from a second component, D_G^a , which arises from the basic fermions being coupled to gauge fields, the so-called 'axial anomaly', $D_G^a = g^2 \bar{\psi} \gamma_\mu F_{\mu\nu}^a \psi = g^2 G_{\mu\nu\lambda\rho} F_{\lambda\rho}^a \psi$ (refs 8, 18, 19). (It is called an anomaly because its occurrence took the theorists by surprise. First discovered by explicit evaluation of simple Feynman graphs involving closed fermion loops, such as triangles, the consequent relation between short and long range behaviour is now understood²⁰ as reflecting a profound topological invariance of the gauge fields and to furnish a particular instance of the Atiyah-Singer index theorem²¹). In the above formula, the $F_{\mu\nu}^a = F_{\mu\nu}^G$ are the gauge fields associated with the gauge potentials, A_μ^a . Each physical gauge field has its own anomaly; for example, the electromagnetic anomaly, $D_G^{\text{EM}} = e^2 \mathbf{E} \cdot \mathbf{H}$, with $\mathbf{E}$ and $\mathbf{H}$ the ordinary electric and magnetic fields, seems to control $\pi^0 \rightarrow \gamma\gamma$ decay^{18,19} (incidentally affording important confirmation of the conjectured existence of the concealed colour degree of freedom). Likewise, in unified gauge theories of the weak and electromagnetic (WEM) interactions, the resultant D_G^{WEM} anomalies are required to cancel if the good high energy behaviour ('renormalisability') is not to be spoiled (see ref. 8). In conventional unified (WEM) schemes, this entails the remarkable prediction that the number of quark types ('flavours') should equal twice the number of lepton types. Counting each changed lepton and its associated neutrinos as one type, this is a prediction with which experiment seems very satisfactorily to have kept pace: $2 \times N(e, \dots) = N(u, d, c, s, t, b, \dots)$. In conventional models, the strong (S) anomaly, D_G^S , is a flavour singlet. It was at first thought to be relatively innocuous¹⁷, in particular, incapable of clarifying the puzzle with the η mass referred to in the previous paragraph.

The next twist to the story concerns the crucial discovery of 'instanton' effects^{22,23} in non-Abelian gauge field theories (the adjective 'non-Abelian' signifies that the corresponding gauge transformations do not all commute). A weak foretaste of this complication is already present in the paradigm of gauge theories, quantum electrodynamics, (QED). There, although the interaction is invariant under the combined U(1) gauge transformations $|e\rangle \rightarrow \exp(-ie\Lambda(x))|e\rangle$ of the electron state vector and $A_\mu^{\text{EM}}(x) \rightarrow A_\mu^{\text{EM}}(x) + \partial\Lambda/\partial x_\mu$ of the electromagnetic potentials, $A_\mu^{\text{EM}}(x)$, gauge dependent quantities do feature in individual contributions to the interaction and in the standard quantisation procedures. (The circumstance that contributions from individual Feynman diagrams in QED are liable to be non-gauge invariant is familiar to particle physicists). In an Abelian gauge theory such as QED, this is merely an inconvenience; in a non-Abelian theory like $\text{SU}(3)^{\text{colour}}$ (QCD), it introduces an essential qualitative enrichment of the structure. The reason is that whereas in a simple gauge theory like QED all groups are topologically continuous, a scheme such as QCD admits topologically disjoint gauge configurations²²⁻²⁵. Configurations of the gauge field potentials, $A_\mu^G(x)$, which arise solely from gauge transformations (for example, $A_\mu^{\text{EM}}(x) = \partial\Lambda^{\text{EM}}/\partial x_\mu$) have zero energy and are thus identified with the vacuum in the quantum version of the theory. The existence of topologically disjoint gauge transformations implies an infinite non-trivial degeneracy of the quantum vacuum. Moreover, these seemingly disjoint gauge vacua do communicate—by

instantons—leading to a redefinition of the vacuum and a whole potential range of new physical effects, welcome and unwelcome, among the latter the threat^{1,2} of unregulated strong PT violation. As earlier with the Nambu-Goldstone-Higgs mechanism, the idea that we have to regard the quantum field-theoretic vacuum as a medium is very much the message.

The 'instantons' are localised non-perturbative solutions of the classical gauge field equations in Euclidean 4-space which connect topologically disjoint vacuum gauges ($F_{\mu\nu}^G = 0$) (the fourth dimension corresponds to imaginary time in an analytic continuation). They have finite classical 'action', $S^{\text{class}} \equiv \int d^4x \mathcal{L}^{\text{class}} = -8\pi/g^2$. It is a general result of quantum theory that classical 'paths' in imaginary time correspond to quantum tunnelling; in the present context, this tunnelling associates the apparently disjoint gauge vacua $|\Psi_n\rangle = |n\rangle$ into new superpositions, $\Psi_\theta = \sum \exp(in\theta)|n\rangle$ defined by a phase angle, θ whose value is not as yet specified by theory²³⁻²⁵. The crucial link of these considerations with chiral symmetry breaking comes from the observation²³ that the index n which labels the topologically distinct configurations of the gauge field is controlled by the axial anomaly, $n = \frac{1}{2}i \int d^4x D_G^a$. The way instanton effects feed into the rest of the dynamics is expressed in the modified formula^{24,25} for the evolution of the total quantum system (technically, the vacuum to vacuum transition amplitude) for the ' θ -vacuum' which is given by $\langle 0|0\rangle_\theta = \sum_n \int (\text{fields}) \exp[\int \text{Lagrangian}] \times \exp(in\theta)$. It is just as if an extra contribution $\frac{1}{2}\theta \cdot D_G^a$ were added to the original interaction.

The quantity θ thus has the role of an additional physical parameter, an extra coupling constant not present in the original specification of the dynamics^{1,2}. As it is associated with a pseudoscalar quantity, it induces PT violation. From the defining equations, it has the rather unusual cyclic property of being invariant against translations of 2π . In practice, it has to be very small ($\theta \approx 3 \times 10^{-5}$) in order not to yield a contribution to neutron electric dipole moment in excess of the observed experimental limit²⁶.

This defines the problem which the proposed enlargement of the Higgs couplings⁹, and the consequent prediction of axions^{1,2}, was designed to counter. Two complex Higgs doublets do the work usually assigned⁸ to the single doublet ϕ and its complex conjugate $\phi^\dagger$. Provided at least one quark acquires its mass from the Higgs coupling, this introduces an additional U(1) chiral symmetry sufficient to ensure that $\theta \approx 0$ should emerge automatically, in keeping with the naturalness maxim referred to above. The axions are the Goldstone bosons associated with the new symmetry^{1,2,12}.

If we suppose that axions do not exist, then we have to re-adjust our concept of what is a 'natural' theory is in order to accommodate nature. Ellis and Gaillard examined the implications of axions not existing and questioned the original motivation for introducing them. They argue, that it is a property of our world that $\theta^{\text{strong}} \approx 0$; furthermore, there is no question of this result being renormalised by the strong interactions. They have also shown using a representative model for (WEM) couplings that weak and electromagnetic interactions likewise produce no corrections in low order; any residual effects should therefore be compatible with the experimental neutron electric dipole limit. Ellis and Gaillard further emphasise that the effective range of instanton effects, which is controlled by the requirement that the associated 'action', $\exp(-8\pi/g^2(\mu^2))$ be appreciable ($\mu \approx 500$ MeV), is at variance with the very short range $0(M_\pi^-)$ commonly ascribed to CP (PT) violation.

The present conclusion is then that axions probably do not exist, and interest among gauge theorists has reverted to the study of other potentially very important consequences of the non-perturbative topological transitions of gauge fields (instantons and the like); in particular, to exploring the bearing of these phenomena on the outstanding problem of colour confinement. However, should the experimenters have second thoughts, the theorists will clearly be ready.

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articles

Fast and slow myosin in developing muscle fibres

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Slow and fast isoenzymes of myosin coexist in all the fibres of a fast-twitch mammalian muscle during early development. They later become segregated into different populations of fibres. Slow myosin is most abundant when the speed of contraction of the muscle is slow and the fibres are multiply innervated; its synthesis in the majority of the fibres seems to be 'switched off' when the speed of contraction increases and the fibres become innervated by single motoneurons.

DURING the early development of skeletal muscles, the speed of contraction tends to be slow, even in those muscles which contract rapidly in the adult animal¹⁻⁴. However, myosin from future slow as well as fast muscles contains light chains with electrophoretic mobilities comparable to those of adult fast muscles⁵⁻⁸. In addition, fibres with a high level of myosin ATPase activity predominate in differentiating muscles⁹⁻¹⁴. Actomyosin ATPase activity of whole muscles has been shown to be directly proportional to speed of contraction in the adult¹⁵. Because of the apparent contradiction between chemical and physiological data, it has been suggested that speed of contraction is not related to the type of myosin or the type of fibre present in developing muscle^{16,17}. Using an immunocytochemical approach, we have demonstrated that significant amounts of slow as well as fast isoenzymes of myosin are present during early development of the rat diaphragm, a fast-twitch muscle. Moreover, these isoenzymes coexist in the same fibres.

Localisation of myosin isoenzymes

The anterior latissimus dorsi (ALD) of the chicken is widely used as an example of a slow red muscle. Myosin extracted from this muscle and purified by ion-exchange chromatography (ref. 18 and G.F.G. and S.L., in preparation) was used as an antigen to elicit antibodies in the rabbit against 'slow' myosin. However, a small population of fibres from the ALD contain

white ('fast') myosin^{18,19}, which could contribute to the immunogen. To remove such contaminating antibodies, the serum was passed through an immunoabsorbent column composed of myosin extracted from a region of the pectoralis which consists entirely of white fibres¹⁸. Antibodies specific for white myosin would be bound to this column, whereas the unretained fraction would presumably represent antibodies specific for red ('slow') myosin (G.F.G. and S.L., in preparation). The specificity of this adsorbed serum was shown by its ability to react with frozen sections of chicken muscle. As expected, it reacted strongly with the anterior latissimus dorsi, but failed to react with either the posterior latissimus dorsi (PLD) or the pectoralis—both fast muscles. In some experiments the adsorbed serum was further purified by passage through an affinity column of Sepharose coupled to ALD myosin. The bound antibody was eluted with 4 M guanidine hydrochloride and rapidly dialysed free of denaturant. The results obtained with this specific antibody were identical to those obtained for the immunoglobulin fraction of the adsorbed serum.

The myosin of a fast-twitch muscle, the chicken pectoralis, contains two chemically different classes of light chains. One class can be isolated by means of the thiol reagent DTNB, and the other is released on exposure of myosin to alkaline pH, hence the name 'alkali light chains'. There are two types of alkali light chains, which have extensive sequence homology, but the larger alkali 1 (21,000 molecular weight) has an additional 41 N-terminal residues which are absent in alkali 2 (17,000 molecular weight)²⁰. Recently an antibody specific for this sequence, termed the 'difference peptide' ($\Delta 1$), has been isolated from anti-alkali 1 sera and purified by affinity chromatography²¹. An antibody has also been isolated which is specific for a small sequence ($\Delta 2$) unique to alkali 2 (refs 22,23). The two alkali light chains of adult myosin reflect isoenzymes which are predominantly homodimers²¹, that is, myosin molecules containing 2 mol of either alkali 1 or alkali 2 light chains. Here we shall refer to myosin which reacts with anti- $\Delta 1$ or anti- $\Delta 2$ as A1 myosin or A2 myosin, respectively. This designation does not imply that myosin in embryonic muscles is necessarily the same as in adult muscles.

Transverse cryostat sections of rat diaphragm were exposed to these antibodies and subsequently reacted with fluorescein-labelled goat antibodies against rabbit immunoglobulin. Fibres which have reacted with the antibodies appear bright when examined with a fluorescence microscope. The diaphragm was studied at various stages of development from 19 days gestation to 30 days postnatal. At each stage, the muscle was sectioned simultaneously with a specimen of adult diaphragm in the same block. In this way, differentiating fibres can be compared directly with adult fibres of equal thickness in identical experimental conditions.

In the adult diaphragm, four types of fibres can be identified by combined histochemical, immunocytochemical and ultrastructural procedures¹⁸. All white, intermediate and about half the red fibres ('fast' red fibres) react with antibodies against fast white myosin as well as the light chains of fast myosin (Figs 1-4a and b) (G.F.G. and S.L., in preparation). The remaining red fibres ('slow' red fibres) are unreactive. Antibodies against slow red myosin react strongly with slow red fibres, but not with fast red or with intermediate or white fibres (Figs 1 to 4c). The pattern of response to anti-slow myosin in the adult muscle, therefore, is reciprocal to that observed with antibodies against fast myosin.

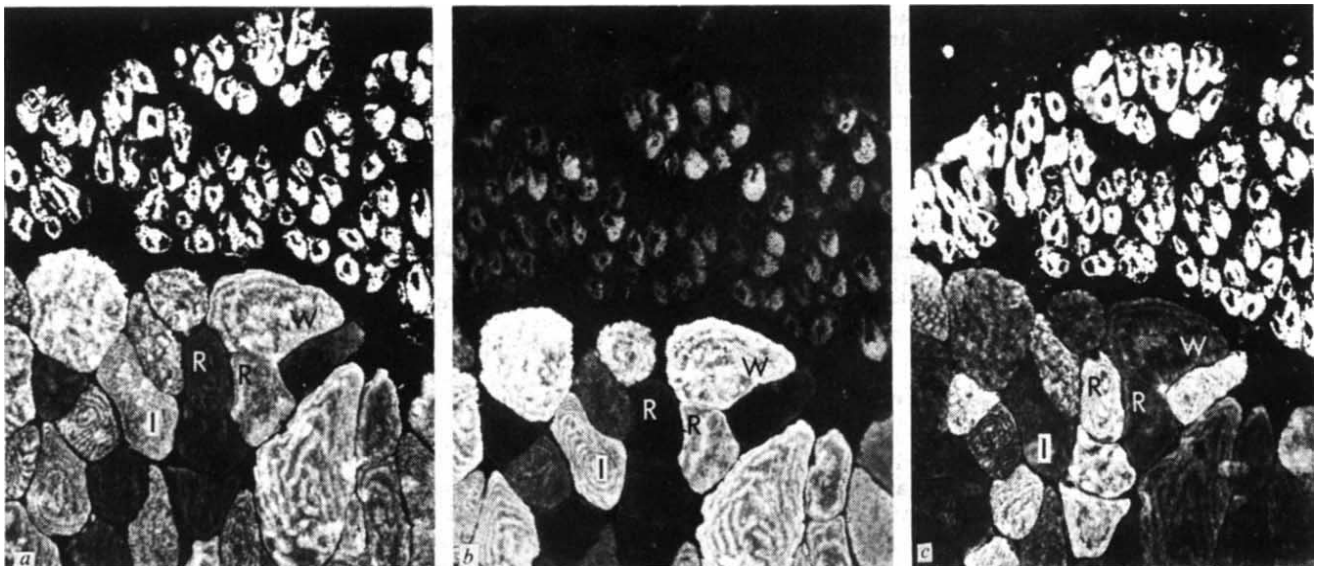
Up to the third week of postnatal development, all fibres in the rat diaphragm react with antibodies to both fast and slow myosin (compare Fig 1a and c with Fig. 4a and c). At 19 days gestation, when the fibres are still in the myotube stage, all fibres react strongly with antibodies against slow myosin (anti-ALD) as well as the alkali 1 light chain (anti- $\Delta 1$) of fast myosin (Fig. 1a, c). The response is even greater than in adult reacted fibres. The same fibres react with antibody specific for the alkali 2 light chain (anti- $\Delta 2$) (Fig. 1b), but the response is far less intense than with anti- $\Delta 1$. On postnatal days 1 and 7 a differential response to anti- $\Delta 1$ and to anti-ALD myosin is evident; most of the fibres continue to stain intensely with

anti- $\Delta 1$, but some are only moderately reactive (Figs 2a and 3a). A minority of the fibres react strongly with anti-ALD, and the majority are moderately reactive when compared to unreacted fibres of the adult (Fig. 2c). Staining is somewhat less at day 7 (Fig. 3c) than at day 1. Some fibres react strongly with both anti- $\Delta 1$ and anti-ALD myosin, and the overall level of response to anti-ALD myosin within the fibre population is still greater than in the adult muscle. All fibres continue to react with anti- $\Delta 2$ (Figs 2b and 3b); staining has increased to adult levels, but it is still less than with anti- $\Delta 1$. By 21 days there is a differential response to anti- $\Delta 2$ (Fig. 4b). In addition, the positive response to anti- $\Delta 1$ has now decreased to the adult level (Fig. 4a). The majority of the fibres at this stage fail to react with anti-ALD (Fig. 4c), and the pattern of response has become reciprocal to that seen with antibodies against the light chains of fast myosin. The response to all three antibodies is, therefore, comparable to that observed in the adult fibre population.

In these studies we have used antibodies against the N-terminal sequences of the alkali 1 and alkali 2 light chains, because these are the only available antibodies capable of distinguishing between the two isoenzymes of fast skeletal muscle myosin. However, these antibodies give no direct information about the localisation of the heavy chain of fast myosin. We therefore reacted developing muscles (19 days gestation and day 1 postnatal) with antibodies specific for the rod region of myosin (G.F.G. and S.L., in preparation) and with anti-pectoralis myosin absorbed with total light chains. The pattern of fluorescence obtained with these antibodies was the same as that observed with antibodies to the light chains.

At all developmental stages examined, the demonstration of both A1 and A2 myosin and slow myosin by immunofluorescence correlates well with the localisation of ATPase activity in the same fibres. In adult muscles, the ATPase of a particular fibre is stable after either alkali or acid preincubation, but

Fig. 1 Rat diaphragm, serial transverse sections; 19 days gestation. Antibodies against a, A1 myosin (anti- $\Delta 1$); b, A2 myosin (anti- $\Delta 2$); c, slow myosin (anti-ALD). Developing muscle (upper) was sectioned simultaneously with a specimen of adult diaphragm (lower) so that differentiating fibres can be compared directly with adult fibres of equal thickness in identical conditions. Although intensity of fluorescence fades during exposure to ultraviolet light, the adult muscle provides a reference against which the relative intensity at each stage of development can be compared. The same fibres are given the same designations in each section. Adult fibre types were classified on the basis of mitochondrial content, demonstrated by localising succinic dehydrogenase activity in sections serial to those reacted with labelled antibodies. Fast fibres were identified by a high level of alkali-stable ATPase activity in comparable sections. Note that antibodies against the light chains of fast myosin (a and b) react only with white (W), intermediate (I), and fast red fibres (black R) of the adult muscle (lower). Antibody against slow myosin (c) reacts with slow red fibres (black R); an occasional fast red fibre may react as well. All fibres in the developing muscle (upper) react strongly (greater than the adult) with both anti- $\Delta 1$ (a) and anti-ALD (c) and weakly (less than the adult) with anti- $\Delta 2$ (b). $\times 225$.



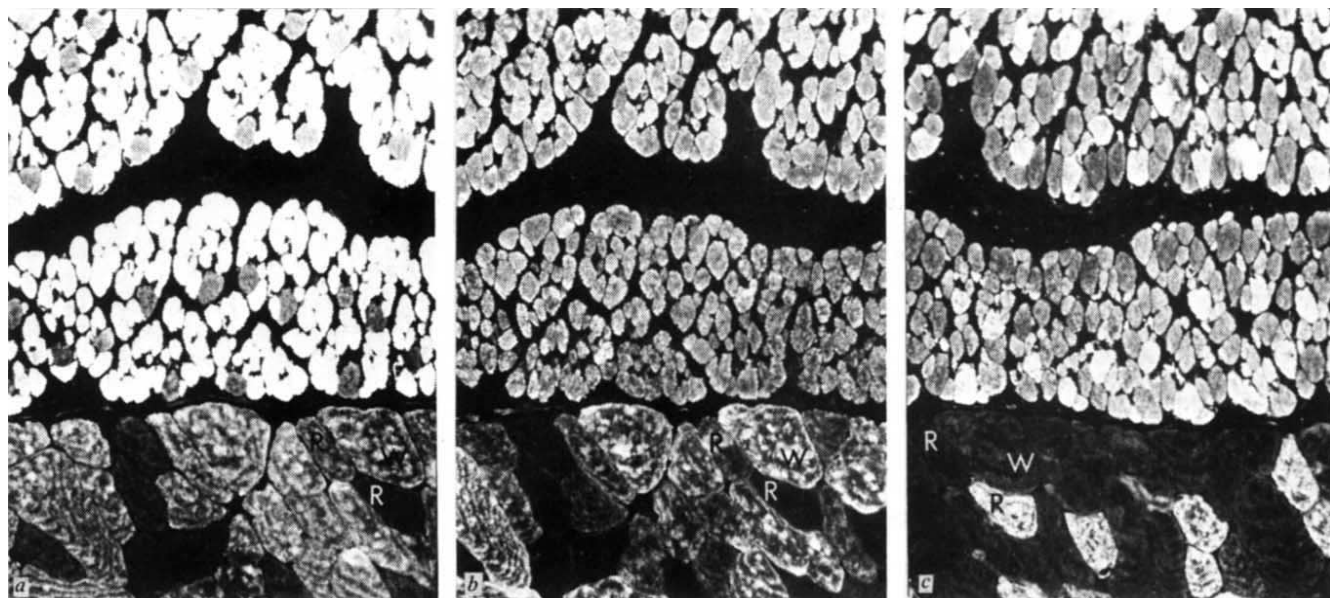


Fig. 2 Rat diaphragm, serial transverse sections; day 1 postnatal. There is a differential response to anti- $\Delta 1$ (a) and anti-ALD myosin (c). Most fibres stain intensely with anti- $\Delta 1$, but some are only moderately stained (a). A minority of the fibres are stained intensely with anti-ALD, and the majority are stained moderately (c); the overall response of the fibre population continues to be greater than in the adult muscle (lower). The response to anti- $\Delta 2$ is the same in all fibres (b), but the level has increased. $\times 225$.

usually not both²⁴. In the adult rat diaphragm, fibres which react with antibodies specific for fast myosin have an alkali-stable ATPase, whereas those fibres which react with antibodies against slow myosin have acid-stable ATPase (G.F.G. and S.L., in preparation). At 19 days gestation, when both fast and slow myosin coexist in all fibres, the same fibres have ATPase activity which persists after either alkali or acid preincubation. During the first week of postnatal development, there is a difference in the level of alkali-stable ATPase activity among fibres, and the pattern resembles the distribution of A1 myosin demonstrated by immunofluorescence. The distribution

of acid-stable ATPase resembles that of slow myosin, being highest in a minority of the fibres.

Physiological implications

The overall pattern of change in the distribution of myosin isoenzymes is consistent with physiological changes that occur in mammalian skeletal muscles. Adult contractile properties are gradually acquired during late prenatal and early postnatal development, the exact time depending on the species and on the specific muscle analysed^{1,2,12}. In the rat diaphragm, during a

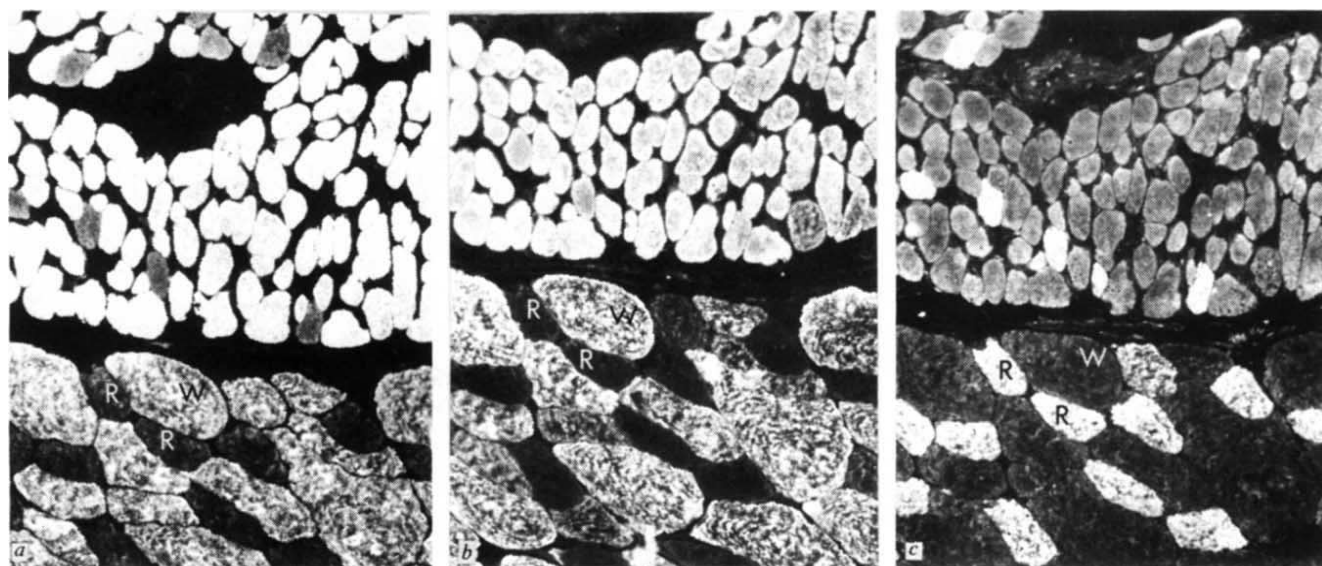


Fig. 3 Rat diaphragm, serial transverse sections; day 7 postnatal. The differential response to anti- $\Delta 1$ (a) and anti-ALD (c) is evident, but the response to anti- $\Delta 2$ (b) is still the same in all fibres. Although the level of response to anti-ALD is somewhat lower than at day 1 (Fig. 2c), the overall intensity remains greater than in the adult muscle (lower), and some fibres react strongly with both anti- $\Delta 1$ and anti-ALD. $\times 225$.

period when the rate of contraction is slow (up to 7 days)²⁵, slow myosin is present along with fast myosin. A2 myosin is present, but is relatively less abundant than A1 myosin. By 21 days, when the speed of contraction has increased in the same muscle, the overall level of staining for slow myosin has decreased. Furthermore, preliminary observation of a fast hind-limb muscle of the rat, the extensor digitorum longus (EDL), indicates that, at postnatal day 1, slow myosin and A1 myosin predominate, and A2 myosin is present in lesser amounts (Fig. 5*a, b, c*). Speed of contraction in the EDL is slow at this time². Hence, the presence of slow myosin in the developing diaphragm is not unique. Our observations suggest, therefore, that speed of contraction is inversely related to the amount of slow myosin and perhaps directly related to the amount of A2 myosin present during development. There is some evidence to suggest that the alkali 2 light chain may be associated with fast contraction, because myosin subfragments containing the alkali-2 light chain have a higher actin-activated ATPase activity than those containing alkali 1^{26,27}. In addition, the ATPase activity of myosin from developing muscle is somewhat lower than that of the adult^{6,7,28}.

It has been reported that the alkali 2 light chain is not present during the early development of chicken fast muscle^{17,29}. However, we have found that some A2 myosin occurs in all fibres of the rat diaphragm during early development. This apparent discrepancy may be related to a difference in species or to the time of innervation. In the rat diaphragm, even before 19 days gestation, synapse formation has already taken place³⁰, and we have observed A2 myosin at 19 days in the same muscle. In the pectoralis muscle from an 11-day chicken embryo, the A2 light chain was not observed, and in another fast muscle (PLD) from the chicken, innervation is established only after 12 days³. However, lack of innervation alone does not explain the absence of the A2 light chain, since it is also absent in the 20-day chicken embryo¹⁷.

Data on slow myosin are likewise conflicting. We have shown an abundance of slow myosin in two developing fast mammalian muscles, the rat diaphragm and EDL. Similarly, in avian fast muscle, Masaki and Yoshizaki³¹ have demonstrated the presence of slow myosin by the reaction of frozen sections of 10-day embryonic chicken pectoralis with fluorescein-labelled

antibodies against ALD myosin. However, Rubinstein, Pepe and Holtzer¹⁷ have reported a complete absence of slow myosin in 11-day embryonic chicken pectoralis by both electrophoretic and immunological techniques. Some of these discrepancies may arise from the practice of identifying embryonic myosin as A1 myosin on the basis of the electrophoretic mobility of its light chains on SDS gels. There is evidence, from two-dimensional O'Farrell gels, for an embryonic light chain which is distinguishable in charge from the alkali 1 light chain, but has the same molecular weight (R. G. Whalen, G. S. Butler-Browne and F. Gros, in preparation). Furthermore, one must recognise the possibility that some fast myosin alkali light chains may, during development, be associated with heavy chains of the slow type. In that case, it may be an oversimplification to attempt to relate any embryonic myosin to an adult type on the basis of its light chains, be it by electrophoretic or immunological methods. Until the heavy chains of embryonic myosin have been characterised by some form of peptide analysis³², it will not be possible to give a definitive description of the type and number of myosin isoenzymes present in a developing muscle.

Relationship to innervation

Contractile activity during development is clearly influenced by the nervous system. Change in speed of contraction occurs abruptly once innervation is established in avian muscles, and gradually following innervation in mammalian muscle. In both avian and mammalian muscles, the normal differentiation of contractile activity can be altered by denervation or cross-reinnervation during early development^{1,3}. This is consistent with evidence that both the contractile properties and the type of myosin of adult muscles are influenced by the type of nerve supply. It is possible, for example, by cross-reinnervation with a 'slow' nerve or by prolonged stimulation, to convert a fast into a slow muscle and likewise to transform the properties of the myosin³³⁻³⁷. Recent physiological studies have shown a dramatic shift in the pattern of innervation during the first 3 weeks of postnatal development in mammalian skeletal muscles. Through the first week, each muscle fibre receives axons from two or more motoneurons^{30,38-41}. This 'poly-

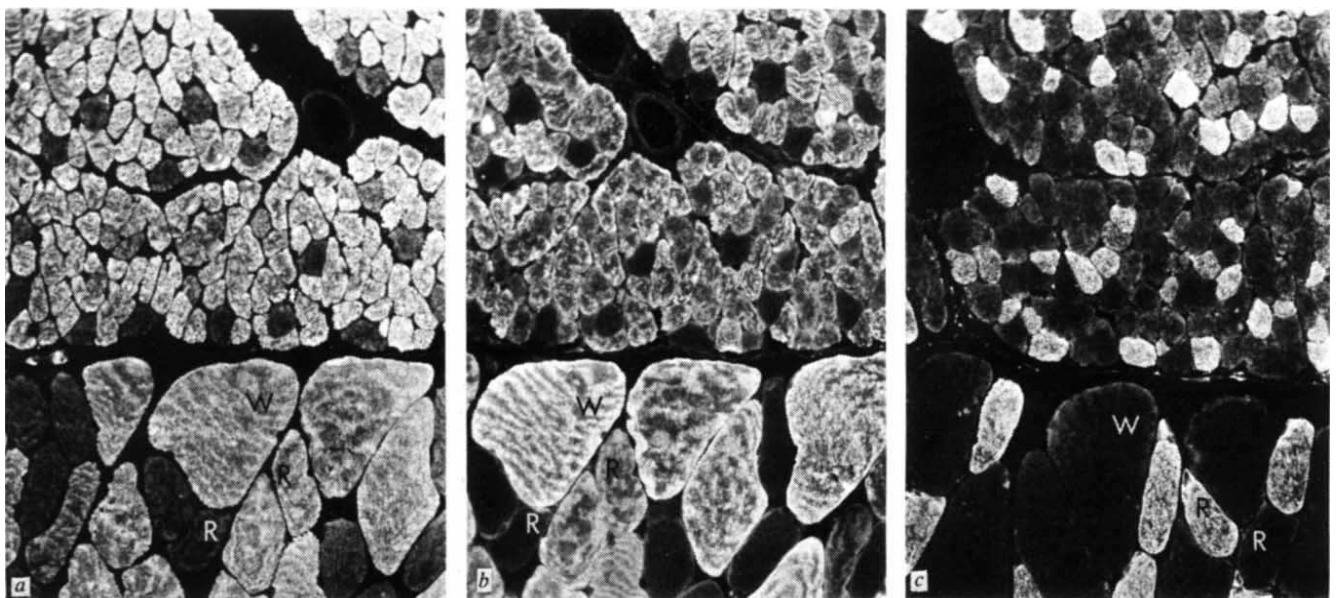


Fig. 4 Rat diaphragm, serial transverse sections; day 21 postnatal. A differential response to anti- $\Delta 2$ is now evident (*b*). The positive response to anti- $\Delta 1$ in the majority of the fibres is now equivalent to the adult (*a*), and the majority of the fibres fail to react with anti-ALD (*c*). The overall response to all three antibodies is comparable to the adult muscle (lower) in each case, and the pattern of response to anti-ALD (*c*) is reciprocal to that seen with antibodies against the light chains of fast myosin (*a, b*). The section in (*c*) is not serial to that shown in (*a*) and (*b*) in this illustration. $\times 225$.

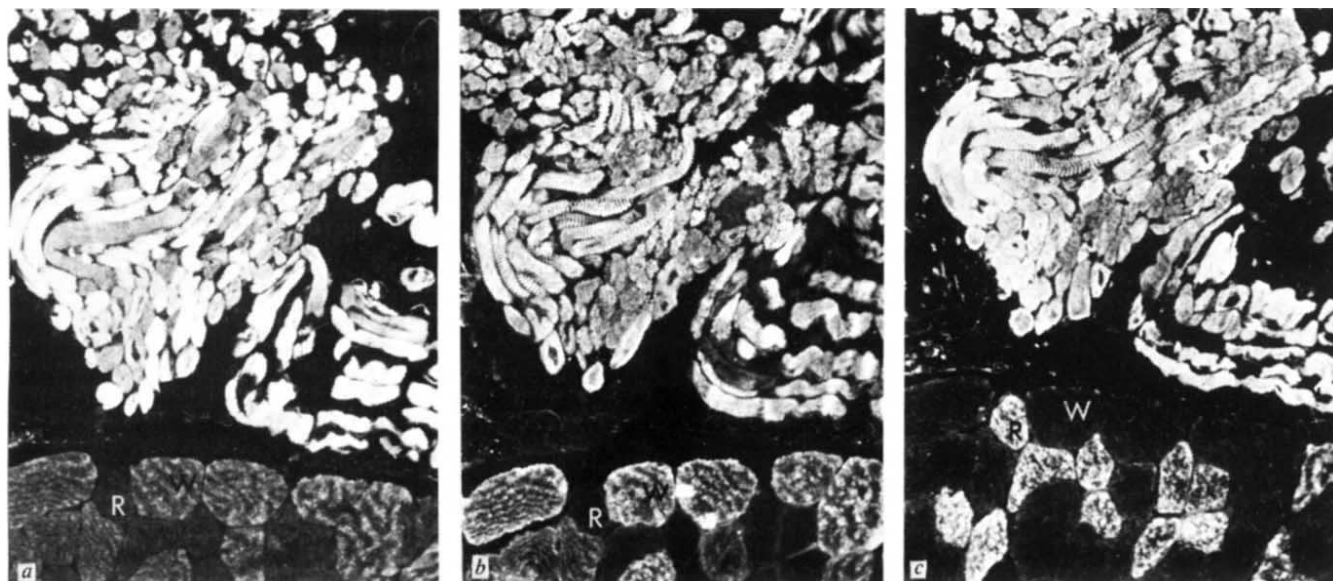


Fig. 5 Rat EDL, serial sections adjacent to adult diaphragm; day 1 postnatal. Many of the fibres in this preparation are sectioned longitudinally, and transverse striations reflect localised reaction of antibodies with A-band regions. Distribution of myosin isoenzymes is similar to that observed in the diaphragm muscle during early development. Most fibres react strongly with both anti- $\Delta 1$ (a) and anti-ALD myosin (c) and moderately with anti- $\Delta 2$ (b). Hence slow as well as fast myosin is widespread in this future fast-twitch muscle, and the presence of slow myosin in the diaphragm is not unique. $\times 255$.

neuronal innervation' is slowly withdrawn until each muscle fibre is innervated by only one motoneurone. Our evidence indicates that slow and fast isoenzymes of myosin coexist within individual fibres, and this correlates well with the period of polyneuronal innervation. In the rat diaphragm, multiple innervation of a single muscle fibre by several motoneurons is widespread at 19 days gestation³⁰. We have shown that, at this time in the same muscle, all fibres stain intensely with antibodies against slow myosin and the alkali 1 light chain of fast myosin and moderately with antibody against the alkali 2 light chain. At days 1 to 7 postnatal, the incidence of multiple innervation is still high, but it has begun to decrease. Slow myosin and A1 myosin are now becoming segregated into different populations of fibres. That is, differences in fibre type are now becoming evident. However, all fibres still contain some slow myosin and A1 myosin, and the uniform distribution of A2 myosin persists. At 21 days, all fibres are innervated by single motoneurons, and the differential pattern of distribution of all three isoenzymes of myosin, including A2 myosin, is comparable to that of the adult. The fibre type pattern characteristic of the adult rat diaphragm has now been established.

In conclusion, using highly specific antibodies, we have been able to localise different isoenzymes of myosin with respect to the fibre population during various stages in the development of a fast-twitch mammalian skeletal muscle. We have shown that both fast and slow types of myosin are present within the same fibres and that, at the earliest time intervals, slow myosin is more widespread than in the adult muscle. These findings seem to correlate with a slow rate of contraction during development. Furthermore, as slow myosin is present in all fibres in the prenatal period, but in only a minority of the fibres in the adult (see Fig. 1c), it is unlikely that the progressive decrease of slow myosin reflects a preferential loss of a particular type of fibre. Rather, the evidence suggests that, in most fibres of a fast-twitch muscle, the synthesis of slow myosin (or an embryonic myosin with characteristics of slow myosin) is 'switched off' during the normal course of differentiation, perhaps in response to withdrawal of multiple innervation of the fibres.

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Extended X-ray absorption fine structure determination of iron nitrogen distances in haemoglobin

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EXAFS spectra have been obtained of oxy and deoxy complexes of haemoglobin and of the 'picket fence' porphyrin, using synchrotron radiation as a source of X rays. The fluorescence data were Fourier filtered to obtain distances to the first shell and corrections applied to remove contributions from the axial ligands. In this way, the iron to porphyrinato nitrogen distances were determined to be $1.98 \pm 0.01 \text{ \AA}$ for both oxygenated complexes and $2.055 \pm 0.01 \text{ \AA}$ for both deoxy forms.

THE high X-ray fluxes available from synchrotron radiation have made it possible to obtain extended X-ray absorption fine structure (EXAFS) spectra¹⁻⁴ in dilute systems including metalloproteins⁵⁻⁸. In this kind of experiment X rays absorbed by the metal atom create photo electrons which when back-scattered by the ligands modulate the X-ray absorption. Simultaneous advances in experimental techniques⁹, data analysis¹⁰, and theoretical understanding¹¹⁻¹⁷ have made it possible to obtain nearest-neighbour interatomic distances with accuracies of $\sim 0.01 \text{ \AA}$, when these bond lengths are the same^{10,17,18}. Preliminary EXAFS spectra of haemoglobin⁸, in which the absorption was measured near the Fe K edge at $\sim 7,100 \text{ eV}$ showed significant differences between oxy-haemoglobin (HbO_2) and deoxygenated haemoglobin (Hb). Furthermore the bond lengths to the iron in the low affinity Hb A and the high affinity Hb Kempsey were shown⁸ to be identical with an accuracy of $\pm 0.02 \text{ \AA}$. In order to determine the actual bond lengths from the spectra two simplifying advances in the data analysis have been developed and are applied here. First, a Fourier filtering technique is used which retains contributions from only the first shell of ligands. Second, corrections are applied to the first shell data to remove contributions of the axial ligands so that we obtain lengths of the iron-porphyrinato-nitrogen (Fe-N_p) bonds. We describe here fluorescence EXAFS measurements on HbO_2 and Hb as well as on the oxygenated (PFO_2) and deoxygenated (PF) forms of $\text{Fe}(\text{TpiVPP})(\text{N-MeIm})$ the so-called 'picket fence porphyrins' which have been synthesised by Collman and his collaborators and shown to be capable of reversible oxygenation (ref. 19 and refs therein). The Fe-N_p distances determined in this study for both HbO_2 and PFO_2 are $1.98 \pm 0.01 \text{ \AA}$ and for both Hb and PF are $2.055 \pm 0.01 \text{ \AA}$. For PFO_2 our results for this distance are shown to be in perfect agreement with the X-ray diffraction studies^{20,21}.

These bond lengths in Hb and HbO_2 have been of considerable interest because of the Hoard²² Perutz²³ hypothesis. Reasoning from crystal structure data on five coordinated ferric haems which had shown Fe-N_p to be $\sim 2.07 \text{ \AA}$ and from the $0.12 \text{ \AA}$ difference between ferrous and ferric observed in ionic compounds, they had proposed that the Fe-N_p bond in Hb would be as long as $2.19 \text{ \AA}$, so that the iron would be forced out of the haem plane. They suggested that the forced iron motion was the major pathway by which the strains of oxygenation was transmitted to the rest of the molecule, and the crystal structure

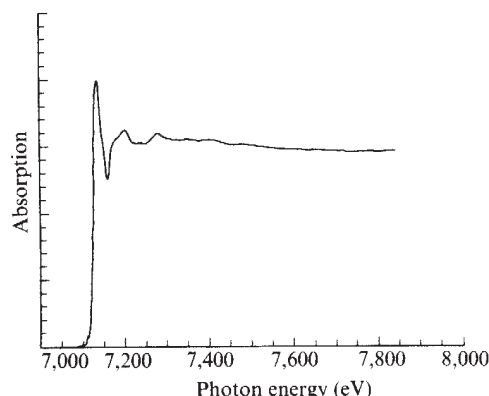
of Hb was interpreted as showing the iron displaced $0.75 \text{ \AA}$ above the haem plane²³. The short Fe-N distances which we have found in Hb are quite close to those determined in recent crystal structures of five coordinated high-spin ferrous haems^{24,25}. It is shown that if the iron is indeed out of the plane of the nitrogen's (and our estimate by triangulation is $0.2^{+0.1}_{-0.2} \text{ \AA}$ for this displacement) it has not been forced out by the Fe-N_p bond, because the strain energies are small compared to thermal energies, nor has it been pulled out by the globin since PF and Hb are identical, but probably it is moved by uncompensated steric hindrance of the axial imidazole ligand.

Experimental system

HbO_2 was prepared as described previously²⁶ and concentrated to 24 mM haem. The solution contained 5% to 10% metHb as measured by optical spectral changes on addition of KCN. During the experiments the degree of oxidation increased from 10% to 20%. For Hb the degree of oxidation was approximately 10% and did not change appreciably after X-ray exposure. Several mg of dithionite were added to the X-ray exposed Hb sample and the measurement was repeated. The Hb Kempsey data were as reported previously⁸. The powdered picket fence samples were prepared by J. P. Collman and T. R. Halbert. The PFO_2 was deoxygenated to give PF. Crystals of bis(imidazole)protoporphyrin IX Fe(III) chloride were prepared by J. Peisach.

The basic experimental procedure for obtaining and analysing the data was as previously described¹⁰⁻¹⁸. A typical fluorescence spectrum for HbO_2 shown in Fig. 1, is the sum of eight runs of 400 points with a total of $\sim 4 \times 10^6$ counts of signal per point. The absorption data are counted as a function of monochromator angle which is then converted into an energy scale. A smooth spline fitted background is then removed and an initial choice of the threshold energy, E_0 , is made which is

Fig. 1 X-ray absorption spectrum of Fe in HbO_2 measured using fluorescence EXAFS techniques. The rapid rise near $7,100 \text{ eV}$ is the Fe K-edge and the small modulation above the edge is the EXAFS.



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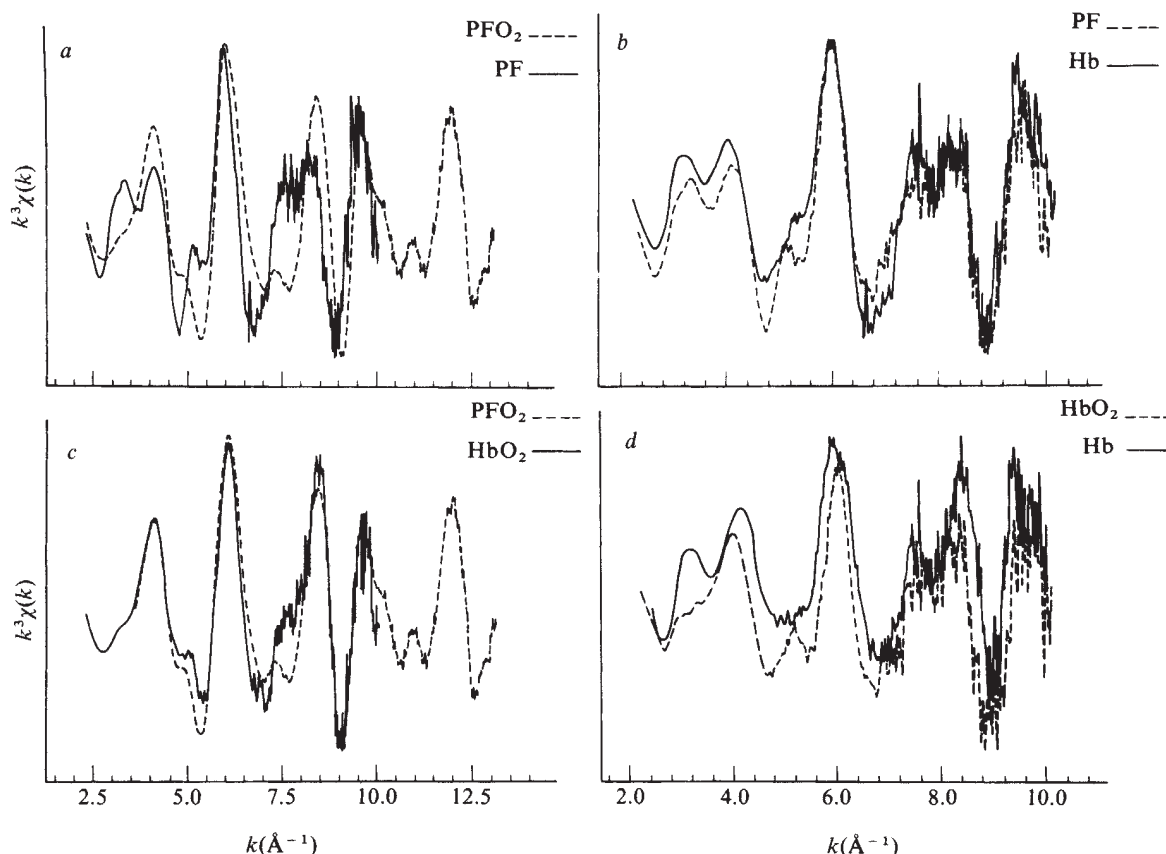


Fig. 2 The oscillating part of the absorption spectra for Hb, HbO₂, PF and PFO₂ after background removal and multiplication by k^3 to equalise the EXAFS amplitudes. The essential point is that Hb and PF are very similar (b and c), and that HbO₂ and PFO₂ are very similar (a and d), while PF and Hb differ greatly from HbO₂ and PFO₂. The analysis described in the text shows that the near-neighbour ligand distances in Hb are the same as in PF, and similarly for HbO₂ and PFO₂.

then used to convert the energy scale to electron momentum, k , by the relation

$$k = \frac{[2m(E - E_0)]^{1/2}}{\hbar} \quad (1)$$

where m is the electron mass. The resultant curve, $\chi(k)$, is then multiplied by k^3 to roughly equalise the oscillation amplitudes. The curve of $k^3\chi(k)$ versus k for HbO₂ is shown in Fig. 2a. The theoretical expression for $\chi(k)$ is¹¹⁻¹³

$$\chi(k) = \sum_i \frac{-N_i |f_i(k, \pi)|}{kR_i^2} e^{-R_i/\lambda} e^{-2\sigma_i^2 k^2} \sin(2kR_i + \delta_i(k)) \quad (2)$$

where the sum is over distinct distances R_i with respect to the absorbing iron atom, where N_i is the number of atoms at R_i , λ is the electron mean free path, $f_i(k, \pi)$ is the back-scattering amplitude, σ_i^2 is the mean squared relative displacement between the iron atom and the scattering atoms from R_i , and $\delta_i(k)$ is the energy-dependent phase shift which depends on the kind of neighbouring atom.

The $k^3\chi(k)$ curves for the four systems studied are shown in Fig. 2. Before any data analysis one can clearly see that Hb is similar to PF and HbO₂ to PFO₂ while very significant changes are observable in going from the oxy to the deoxy forms. These qualitative observations are supported by the quantitative data analysis. The next step in the data analysis is to Fourier transform $k^3\chi(k)$. By applying to the HbO₂ data shown in Fig. 3a the filter shown in Fig. 3b and retransforming back into k space, one obtains the result shown in Fig. 3c. For Fig. 3c only nearest-neighbour contributions are present (the others have been filtered out) and thus equation (2) reduces to

$$\begin{aligned} k^3\chi(k) = & \frac{-4}{kR_1^2} e^{-2R_1/\lambda} k^2 |f^N(k, \pi)| e^{-2\sigma_1^2 k^2} (\sin 2kR_1 + \delta^N(k)) \\ & - \frac{1}{kR_2^2} e^{-2R_2/\lambda} k^2 |f^N(k, \pi)| e^{-2\sigma_2^2 k^2} (\sin 2kR_2 + \delta^N(k)) \\ & - \frac{1}{kR_3^2} e^{-2R_3/\lambda} k^2 |f^O(k, \pi)| e^{-2\sigma_3^2 k^2} (\sin 2kR_3 + \delta^O(k)) \\ & \equiv \sum a_i(k) \sin \phi_i(k) \end{aligned} \quad (3)$$

where the first term corresponds to the four nearest-neighbour porphinato-nitrogens, the second term to the histidyl nitrogen and the third term, in the cases of HbO₂ and PFO₂, to the axial oxygen atom.

If, in retransforming, one also filters out the negative frequencies, then equation (3) becomes

$$k^3\chi(k) = \sum a_i(k) e^{i\phi_i(k)} \quad (4)$$

which can be rewritten in simplified notation as

$$k^3\chi(k) = A(k) e^{i\Phi(k)} \quad (5)$$

In a single distance, single atomic species nearest neighbour system one has

$$\Phi(k) = \phi_1(k) = \tan^{-1} \left[\frac{\text{Im}(k^3\chi(k))}{\text{Re}(k^3\chi(k))} \right],$$

$$A(k) = a_1(k) = |k^3\chi(k)|, \text{ and} \quad (6)$$

$$\Phi(k) = \frac{\pi}{2} + 2kR_1 + \delta^N(k) \quad (7)$$

Hence, if one knows $\delta(k)$ from theory¹⁵ or experiment¹⁰ one can determine R_1 by analysing the total phase function $\Phi(k)$. This analysis was performed here by using a model system with known R_m and assuming chemical transferability of $\delta(k)$ (ref. 10). The function $\Psi(k) = \Phi(k) - \Phi_m(k)$ should equal $2k(R_1 - R_m)$ if no shift exists in E_0 between the two data sets. The function is then fitted by the line $2bk$ with E_0 of the unknown allowed to vary to obtain the best fit. The resultant slope b , together with the known R_m then gives $R_1 = b/2 + R_m$.

This approach has been extensively used^{10,17} and consistently shown to yield 0.01 Å accuracy for systems with known distances. If we use this approach with bis-imidazole as a model for the multiple distance systems and thereby incorrectly neglect the contributions of the axial ligands, we obtain the results given in Table 1 in the column labelled 'before axial correction'. Two things are obvious from these results. First the crystallographically determined Fe-N_p distance in PFO₂ is almost 0.04 Å shorter than the results obtained from the EXAFS data by this crude analysis. Second, within the fitting errors, PFO₂ and HbO₂ have equal distances, as do the three deoxy forms. One can verify this directly by using the picket fence complexes as a model for the respective haemoglobin and then solving for $R_1 - R_m$. This shows that to 0.007 Å accuracy the picket fence porphyrins have the same nearest-neighbour distances as the haemoglobins and as noted before⁸, deoxy Hb Kempsey is the same as deoxy HbA (although now with an accuracy of ± 0.01 Å).

We can, however, go one step further by removing the contribution of the axial ligand to the phase function. Thus we once again reduce the problem to that of a single distance—the Fe-N_p distance. In the three distance case

$$\Phi(k) = \tan^{-1} \left[\frac{a_1 \sin \phi_1 + a_2 \sin \phi_2 + a_3 \sin \phi_3}{a_1 \cos \phi_1 + a_2 \cos \phi_2 + a_3 \cos \phi_3} \right] \quad (8)$$

By using a complex inverse Fourier transform, the filtered data can be uniquely decomposed into $A(k)$ and $\Phi(k)$ for each of the measurements according to the prescription given in equations (6) and (7). Equation (5) can be rewritten in the form

$$k^3 \chi(k) = a_1 e^{i\phi_1} \left\{ 1 + \frac{a_2}{a_1} e^{i(\phi_2 - \phi_1)} + \frac{a_3}{a_1} e^{i(\phi_3 - \phi_1)} \right\} \\ \equiv a_1 a_c e^{i(\phi_1 + \Phi_c)} \quad (9)$$

where Φ_c and a_c are corrections to the phase and amplitude of a single distance.

Table 1 EXAFS Fe-N_p distances

Compound	Before axial corrections	After axial corrections	X-Ray diffraction
PFO ₂	2.016 ± 0.02	1.979 ± 0.01	1.979 ± 0.01* (refs. 20, 21)
HbO ₂	2.029 ± 0.02	1.986 ± 0.01	
PF	2.062 ± 0.006	2.055 ± 0.003	2.069 ± 0.013 (ref. 25)
Hb	2.065 ± 0.003	2.055 ± 0.002	
Hb Kempsey	2.063 ± 0.003	2.053 ± 0.002	

EXAFS Fe-N_p distances (Å) were determined using bis-imidazole as a model³⁰. The errors listed here are fitting errors only. Note that for the two oxygenated complexes, where the axial correction was larger, the fitting errors are smaller after correcting. Because of possible systematic effects, accuracies greater than 0.01 Å should not be taken too seriously. However, direct comparisons which reduce systematic errors between HbO₂ and PFO₂ and between Hb and PF show that the respective distances are equal to within 0.007 Å. Furthermore direct comparisons between oxy and deoxy forms of the same compound give the distance change with oxygenation to be 0.075 Å with an accuracy of ± 0.01 Å. The X-ray diffraction results shown for PF²⁵ were actually made on the polymeric μ -carboxy 'picket fence' where the axial ligand, instead of being histidine, is a carbonyl of the terminal amide of another picket fence haem, leading to linear chains. Our compound, PF, had an *N*-methyl imidazole as the axial ligand.

And

$$\Phi_c = \tan^{-1} \left\{ \frac{\frac{a_2}{a_1} \sin(\phi_2 - \phi_1) + \frac{a_3}{a_1} \sin(\phi_3 - \phi_1)}{1 + \frac{a_2}{a_1} \cos(\phi_2 - \phi_1) + \frac{a_3}{a_1} \cos(\phi_3 - \phi_1)} \right\} \quad (10)$$

In the limit that $\sigma_1 = \sigma_2 = \sigma_3$ and that λ is the same for all first shell atoms, and with the approximation $f(k, \pi) \approx Z/k^2$ for $k \geq 4 \text{ Å}^{-1}$ equation (10) reduces to

$$\Phi_c(k) = \tan^{-1} \left\{ \frac{\frac{R_1^2}{4R_2^2} \sin[2k(R_2 - R_1)] + \frac{8R_1^2}{28R_3^2} \sin[2k(R_3 - R_2) + \delta^O(k) - \delta^N(k)]}{1 + \frac{R_1^2}{4R_2^2} [2k(R_2 - R_1)] + \frac{8R_1^2}{28R_3^2} \cos[2k(R_3 - R_2) + \delta^O(k) - \delta^N(k)]} \right\} \quad (11)$$

Using the axial ligand distance of $R_2 = 2.12 \text{ Å}$ for the deoxy species²⁵ and the theoretical¹⁵ values for $\delta^O(k) - \delta^N(k)$ together with the axial ligand distances of $R_2 = 2.07$ and $R_3 = 1.75$ for the oxy species^{20,21}, one can generate $\Phi_c(k)$ for each measurement assuming a value for R_1 . This $\Phi_c(k)$ is then subtracted from the total phase previously determined from the experimental data such that by equations (8) and (9)

$$\Phi^N(k) = \Phi(k) + \Phi_c(k) \\ = \pi/2 + 2kR_1 + \delta^N(k) \quad (12)$$

where R_1 is the Fe-N_p distance. Having reduced the problem now to one of a single distance, one can then apply the previously described analysis to $\Phi^N(k)$. This was done for various values of R_1 until self-consistency was reached, with the final results given in Table 1 (the column 'after axial corrections'). The previously observed equality among the oxy species and among the deoxy species is still maintained but now the distance in PF agrees with the X-ray diffraction result²⁵ on the similar, but not identical compound to within the combined error limits. PFO₂ is in perfect agreement with X-ray diffraction results on the identical compound.^{20,21}

One might at first be surprised that removing the contribution of the short iron oxygen bond decreased the average Fe-N_p distance. The explanation lies in the fact that we are only fitting the phase function in the momentum region $4 < k < 10 \text{ Å}^{-1}$. In that region the oxygen contribution goes from π to 3π out of phase with the Fe-N_p contribution and this requires the unexpected correction.

Since the correction for the deoxy species is only 0.01 Å it is very insensitive to the initial assumptions including the value of R_2 . Even for the oxy species where the correction is 0.04 Å it does not vary by more than 0.01 Å for sizeable changes in the initial assumptions. The approach used here only attempts to evaluate the change in phase caused by axial ligands and thus involves only differences between the contributions of the axial ligands and the porphyrinato nitrogens. Hence there is less sensitivity to the initial assumptions than in an approach which would attempt to remove the contribution of axial ligands directly from measured spectra using curve fitting.

It is possible that the four porphyrinato nitrogens are not equidistant from the iron. Our results yield values for the average Fe-N_p distance.

Discussion

The basic results of this investigation are given in Table 1, where it can be seen that in HbO₂ and PFO₂ the Fe-N_p distances are $1.98 \pm 0.01 \text{ Å}$ while in Hb, PF, and HbKempsey they are $2.055 \pm 0.01 \text{ Å}$. We shall discuss these results in terms of the proposed pathway by which the effects of oxygenation might be transmitted to the globin. The Hoard-Perutz proposal was based on the distances shown in the first three lines of Table 2. By examination of the measured Ct-N_p distances in

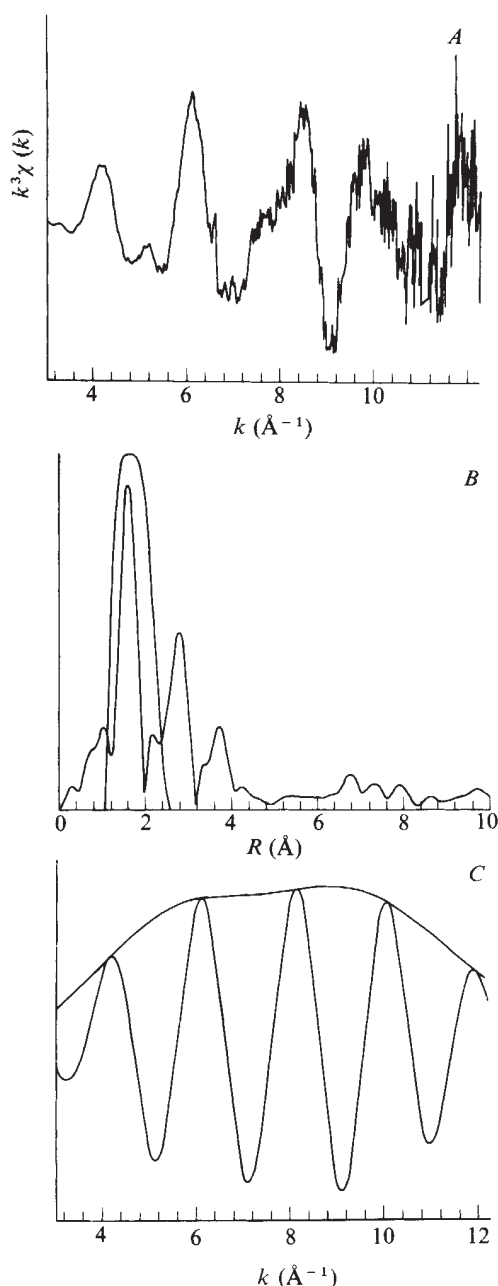


Fig. 3 An outline of the analysis procedure used in this work. A, The EXAFS of HbO₂ as in Fig. 2c and d. The Fourier transform of (A) is shown in (B), where the large peak contains contributions from the 4 Fe-N in-plane ligands and the oxygen and nitrogen axial ligands. The filter window is applied to the transformed data as shown in (B), and the inverse transform computed. The resulting filtered data are shown in (C). The Fourier transform technique also yields the amplitude (shown in C) and phase (not shown) of the filtered data. Further analysis, described in the text, removes the contributions of the axial ligands from the phase function, making it possible to determine the in-plane Fe-N distance to ± 0.01 Å.

three ferric haems, Hoard²² concluded that the relaxed preferred Ct-N_p distance was 2.019 Å. The average Fe-N_p distance in the ferric compounds was 2.073(3) Å. Hoard estimated by analogy with ionic crystals that the ferrous Fe-N_p distances should be longer by as much as 0.12 Å which suggested an Fe-N_p distance of 2.19 Å. The value of 2.14 Å was accepted as being consistent with Ct-Fe = 0.75 Å above the plane which had been reported by Perutz²³ in deoxy haemoglobin. In the present work we see that the Fe-N_p distance instead of being

between 2.14 and 2.19 Å is really 2.055 Å in Hb and also in PF. Is this distance impossible in view of the recent crystal structures of model compounds and in terms of our understanding of the electronic structure of the haem? Since 1971 two crystal structures of five coordinated ferrous haem compounds have been available, that is, the 2-methylimidazole structure²⁴ and that of polymeric μ -carboxy 'picket fence' Fe(II) (ref. 25). The Fe-N_p distances in these compounds, listed in Table 2, are 2.086 and 2.069 Å respectively. Since it is possible that a hindrance to iron movement towards the plane during oxygenation would coexist with a longer Fe-N_p bond, we suggest that the two distances of 2.086 and 2.069 Å previously reported on haems which cannot bind O₂ could be upper limits for those systems like Hb and PF, studied in the present experiments which can reversibly bind oxygen.

Table 2 Structural data based on previously reported X-ray diffraction results of penta-coordinated haems

	Ct-Fe	Ct-N _p	Fe-N _p
Ferric porphyrins ²²	0.48	2.019	2.074
Ferrous porphyrin ²²	0.70	2.02	2.14
Ferrous porphyrin ²³	0.83	2.015	2.18
Polymeric μ -carboxy ²⁵ 'picket fence'	0.28	2.050	2.069(14)
Fe(TPP) (2MeIm) ²⁴	0.42	2.043	2.086 (4)

The first row gives averages of three ferric high spin porphyrins²². The second row shows Hoard's assumed values for ferrous porphyrins²², the third row gives Perutz's assumed values²³. In addition, for the following penta-coordinated porphyrins the average value of Ct-N_p is 2.047 Å. [(H₂O)MgTPP, (H₂O)Zn(TPP), (ClO₄)Zn(TPP), PyZn(TPP) and PyZn(OEP)]²⁴. Ct is the midpoint in the plane of the four porphyrinato-nitrogens, N_p.

Why were the estimated distances of 2.14–2.19 Å for the Fe-N_p bond in high spin ferrous compounds incorrect? They were based on the assumption that ionic radii applied to haem compounds and that on reduction the ferric atom went to ferrous as it does in ionic crystals. However, it had been shown some time ago that for strongly covalent iron compounds with appreciable π bonding such as the iron hexacyanides²⁷ or both high- and low-spin haems²⁸ that the electron introduced on reduction went to the ligands not to the metal and that, in fact, the formal ferrous and ferric ions were actually isoelectronic. Accurate crystallographic data are not available for the iron-carbon bond lengths in ferri- and ferro-hexacyanides. However, good crystallographic data are available for the analogous cobalt hexacyanides and the average Co-C distances are 1.89 Å (ref. 29) for cobalt(III) and 1.90 Å for cobalt(II)^{30,31} respectively. This negligible change on reduction is in agreement with the small changes observed in the five coordinated haem compounds. The average value for the Fe-N_p distance in five coordinated high-spin ferric haems is 2.065 Å and 2.076 Å in ferrous haems. Our results for the ferrous high-spin forms Hb and PF are 2.055 ± 0.01 Å. While it would be more in conformity with chemical experience if the ferrous forms were 0.01 or 0.02 Å larger instead of smaller than the ferric forms we feel that the combined experimental errors do not allow this more usual result and that an explanation of the present results should be sought. Note in Table 2 that the Fe-N_p distance measured by crystallography in the ferric haems equals the average of the ferrous values. The second Hoard assumption is that the relaxed porphyrin would have a Ct-N distance of 2.015 Å (the estimates range from 2.01 to 2.02 Å). Table 2 lists these distances for five coordinated metalloporphyrins. It is clear that the value of Ct-N_p = 2.045 Å which is observed in both high spin ferrous compounds is found generally in iron, magnesium and zinc porphyrins. For a Ct-N_p distance of ~ 2.045 Å our observed value of Fe-N_p = 2.055 Å would fit rather comfortably in the haem plane, while

its maximum compression would be $0.035 \text{ \AA}$ if Ct-N_p were $2.02 \text{ \AA}$. Although a simple Morse potential may not be too accurate since the electronic state can change with geometry³², it can be used to estimate the strain energy in the Fe-N_p bonds associated with the maximum compression of $0.03 \text{ \AA}$. Using a force constant of $0.6 \text{ eV \AA}^{-2}$ it is possible to show that this strain energy is $\sim 10^{-4} \text{ eV}$ which is negligible compared to thermal energy at 300 K . Hence, we conclude that the mechanism proposed by Hoard and Perutz, that is, the iron is forced $\sim 0.7 \text{ \AA}$ out of the plane by its long Fe-N_p bond is not operative in haemoglobin. If $\text{Ct-N}_p = 2.045 \text{ \AA}$ and $\text{Fe-N}_p = 2.055 \pm 0.01 \text{ \AA}$ then at one limit of the Fe-N_p distance the Fe would be in the plane, at the other limit it would be $0.25 \text{ \AA}$ out of the plane. If $\text{Ct-N}_p = 2.02 \text{ \AA}$, which for reasons given we think is an underestimate, the Fe would be $\sim 0.35 \text{ \AA}$ out of the plane. However, in neither case is this geometry, energetically required by the Fe-N_p bond, rather the Fe position is being determined by steric requirements of the axial ligand, now no longer counter balanced by a sixth ligand^{32,33}. In support of that hypothesis our results show that Hb and PF have the same distances so that there is not, on the present scale, any observable bond distortion created by the globin. This is in accord with the position taken by Little and Ibers³⁴ that there are no indications of strain at the iron in Hb. An iron motion of $0.3 \text{ \AA}$ is no larger, and is no more unique than several changes in porphyrin structure observed during oxygenation. The doming of the deoxygenated haem has been shown to involve displacements greater than $0.3 \text{ \AA}$ and has been proposed as a pathway, together with an accompanying haem tilt³⁵. In PFO_2 the short Fe-N_p bonds have buckled the porphyrin ring with a total distortion from planarity of $0.3 \text{ \AA}$ (ref. 21) while twice this distortion has been observed in the low spin Fe(III) (TPP) (bis-Im) complex where the Fe-N_p bonds are also $\sim 1.98 \text{ \AA}$ (ref. 36), and the alternate methine carbons are $0.3 \text{ \AA}$ above and below the plane.

The present EXAFS measurements have accurately determined the average Fe-N_p distances in Hb and PF. We have applied simple triangulation to these distances along with estimates of relaxed porphyrin distances and have estimated that the iron is $0.2^{+0.1}_{-0.2} \text{ \AA}$ out of the plane of the porphyrin nitrogens. The upper limit of $0.3 \text{ \AA}$ does not differ very much from the lower limit $\sim 0.4 \text{ \AA}$ which Hoard²⁴ has pointed out could be an interpretation of Fermi's³⁷ crystallographic study of Hb. However, the point which we make with conviction from the EXAFS results is that the iron is not being forced out

of the plane by very long Fe-N_p bonds. Instead the distortions associated with oxygenation are transmitted through general changes of the haem, rather than being localised in a simple, driven motion of the iron atom.

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Potential for variability through multiple gene products of bacteriophage ΦX174

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The small single-stranded DNA phages ΦX174 and S13 produce multiple products of certain phage genes, as observed by electrophoresis on SDS-polyacrylamide slab gels. Two A protein products, two A products and four G products are observed. The multiple gene products may arise from multiple sites for initiation or termination of translation, or by protein modification. Some of the variant products may provide a substitute for heterozygosity without a concomitant increase in the size of the genome.*

THE nucleotide sequence of bacteriophage ΦX174 is known¹ and provides a unique opportunity for exploring many possible intricacies of the genetic code. The genome of ΦX174 increases its coding capacity in several ways (see Fig. 1). The sequence for gene E is entirely contained within the sequence for gene D but is translated in a different reading frame²; in the same manner gene B is entirely contained within gene A (refs 1, 3), and a newly discovered gene, K, overlaps the terminating codons of B and the 3'-end of gene A and terminates within

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gene *C* (ref. 4). By initiating translation at two different sites in the same reading frame two products of the gene *A* region (*A* and *A**) are made³. If we define a gene by its translation from a unique sequence, then *A* and *A** could be counted as separate genes, but for convenience we will refer to the proteins as multiple products of the same gene. Multiple products could also arise if there were a choice of termination sites.

We show here that several Φ X174 genes specify additional discrete products; gene *G* codes for four proteins and genes *A* and *A** each code for two proteins. In some experiments the closely related phage S13 was studied; its multiple gene products resemble those of Φ X174. The large multiplicity of gene products signifies an extraordinary potential for variability, even from such a small genome.

Identification of four products of gene *A*

Two products of the gene *A* sequence have already been detected, *A* and a smaller protein, *A**, the latter arising by initiation of translation within gene *A* (ref. 5). By increasing the distance the proteins are electrophoresed and by using higher acrylamide and bisacrylamide concentrations, it is possible to resolve each of the two gene *A* products into doublets, *A* into *A*₁ and *A*₂, and *A** into *A**₁ and *A**₂. Lanes 1–8 of Fig. 2 show the positions of the four *A* proteins on fluorograms obtained from two radioactive gels. All four *A* bands are absent when extracts are prepared from cells infected with a 3'-terminal *amber* mutant of gene *A*, Φ X174 *amA29* (lane 3). The same result was obtained for the S13 3'-terminal mutant *amA113*; the proteins *A**₁ and *A**₂ are, however, still made when a nonsense mutation (S13 *amA105*) is located in the

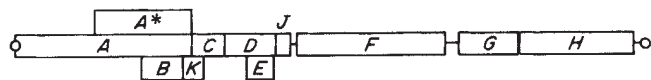


Fig. 1 The circular map of Φ X174 drawn in linear form. The ends are connected where indicated by the small circles, which correspond to nucleotide 3,973 (ref. 1). Translation from left to right.

5'-terminal region of the gene (data not shown). The band separation within each doublet corresponds to about $1,400 \pm 400$ daltons for *A*₁–*A*₂ and 600 ± 200 for *A**₁–*A**₂.

Could the doublets arise because the TGA stop codon that is assumed to end the *A* gene actually allows readthrough about as often as it causes termination? A critical test of this could be provided by nonsense mutants that produce fragments of the *A* and *A** proteins. The doublet pattern would not be seen in such fragments. But except for one case, all nonsense mutants in gene *A* exhibit no *A* fragments at all. The one exception is *amA62* (ref. 3), which can be inferred to be a TGG→TAG mutation six codons before the TGA terminator⁸. The slightly shortened *A* and *A** polypeptides from *amA62* infection (Fig. 2, lane 7) remain doublets, and each component of each doublet has about the same relative abundance as in the *amA*⁺ condition (lanes 6 and 8). Thus, the *A* and *A** doublets are apparently not caused by a combination of readthrough and termination; additional initiation sites or post-translational modification should be considered as possible sources of the doublets.

The possibility remains that the observed *A* and *A** bands are all readthrough products. The *amA62* mutation would shorten the *A* protein by six amino acids (708 daltons) if termination of the wild type occurred at the TGA at nucleotide 134, and by 28 amino acids (3,400 daltons) if readthrough was the rule and termination occurred at the TAA at nucleotide 200. The observed shortening (Fig. 2, lanes 6–8) is calculated to be 980 ± 100 daltons for *A* and 740 ± 100 for *A**. Even allowing for the unreliability of size measurements by gel electrophoresis, our results are more consistent with gene *A* termination at nucleotide 134.

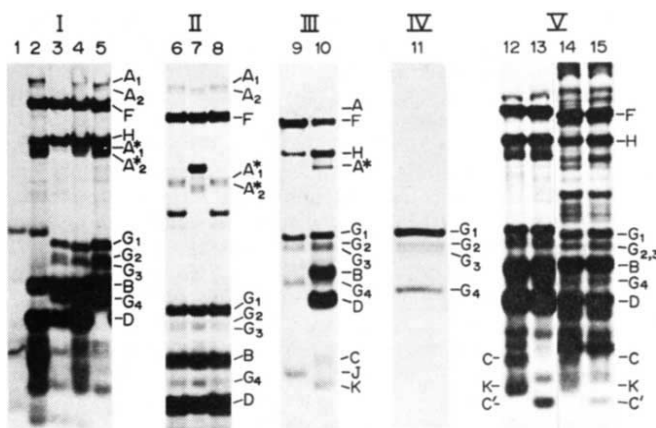


Fig. 2 Fluorograms of ³H-labelled phage-induced proteins after sodium dodecyl sulphate-polyacrylamide gel electrophoresis⁶ (18% w/v acrylamide, 0.14% w/v *N,N'*-methylene-bisacrylamide). Migration is downward. **Gels I, II**, proteins synthesised during phage infection. Cell cultures were labelled with 25–50 μ Ci ml⁻¹ ³H-lysine from 15 to 45 min after infection of ultraviolet ray-irradiated (160–200 J m⁻²) *Escherichia coli* C strain AP1 (Hcr⁺ Sup⁺) and cell extracts were made as described previously⁶. Uninfected cells were treated in a parallel manner. To improve the resolution of the *A* bands in gel II, electrophoresis was for a longer time and the low molecular weight proteins ran out of the gel. Lanes: 1, uninfected cells; 2, Φ X *amG9*; 3, Φ X *amA29*; 4, Φ X *amE3*; 5, Φ X *amD81*; 6, 8, Φ X *amH1*; 7, Φ X *amA62*. Φ X *amH1* was used as the *amA*⁺ control to remove the thick H band from one of the regions of interest. **Gels III, IV**, proteins found in mature phage particles and 6S aggregates. For isolation of phage particles, strain AP1 was grown to 5×10^8 cells per ml in M9 medium and multiply infected with S13 *amE15*. Samples of 5 ml were labelled with 200 μ Ci ³H-lysine from 15 to 60 min after infection. The cells were washed once and lysed in 2 ml 50 mM Tris-HCl (pH 8.0), 5 mM EDTA with 1 mg lysozyme and freeze-thawed in a dry ice-acetone bath. DNase I (10 μ g) was added for 20 min at room temperature to reduce viscosity, debris was removed by centrifugation (20,000g, 20 min), followed by dialysis for 12 h against 50 mM ammonium acetate (pH 8.0). The phage were twice purified by CsCl equilibrium centrifugation, then concentrated by centrifugation (Beckman SW41 rotor, 3 h, 38,000 r.p.m.) and resuspended in electrophoresis sample buffer⁶. Preparation of unfractionated extracts of infected irradiated cells was as described for gels I and II. For isolation of the 6S aggregates, strain AP1 was grown to 5×10^8 cells per ml in M9 medium, concentrated 10-fold, treated with mitomycin C for 30 min at 37°C in the dark without shaking, centrifuged and resuspended at 5×10^8 cells ml⁻¹ in fresh M9 medium. Samples of 1 ml were infected with Φ X *amB16* in the presence of 10^{-2} M MgSO₄ and labelled with 50 μ Ci ³H-lysine from 10 to 15 min after infection. Cultures were lysed and extracts sedimented through 5–30% sucrose gradients (SW41, 18 h, 40,000 r.p.m.) as described previously⁷. Fractions were collected dropwise from the bottom of each tube and prepared for electrophoresis by adding a 1/10 volume of 10-fold concentrated sample buffer. Lanes: 9, S13 *amE15* capsid proteins; 10, unfractionated proteins of S13 *am*⁺-infected irradiated cells; 11, 6S aggregate from Φ X *amB16*-infected cells. **Gel V**, proteins synthesised during *ocC* and *ocC*⁺ phage infections. Labelling was as described for gels I and II except Φ X174 infections (lanes 14 and 15) used unirradiated cells. Lanes: 12, S13⁺; 13, S13 *ocC286*; 14, Φ X⁺; 15, Φ X *ocC6*.

Identification of four products of gene *G*

The fluorograms also show multiple *G* proteins (Fig. 2). An extract made from uninfected irradiated cells was electrophoresed in lane 1. The extract of Φ X174 *amG9*-specified proteins in lane 2 lacks four bands found in lane 3 (*amA*), lane 4 (*amE*) and lane 5 (*amD*). The missing bands are designated *G*₁, *G*₂, *G*₃ and *G*₄. The band separation corresponds to approximately 2,000 daltons for *G*₁–*G*₄.

The following observations support the view that the capsid gene *G* (ref. 9) codes for these multiple products. Lysine-labelled S13 and Φ X174 *amE* phage were twice purified by equilibrium-banding in CsCl density gradients and the mature capsids dissociated and denatured for electrophoresis. Both phage-capsid preparations contained, in addition to proteins F, H and J, relative amounts of proteins G₁, G₂ and/or G₃, and G₄ similar to those found in unfractionated infected-cell extracts (Fig. 2, lanes 9 and 10 for S13). The presence of both G₂ and G₃ in the coat could not always be demonstrated with certainty. A 6S aggregate of G protein can be isolated following lysozyme-EDTA treatment of infected cells⁷. We find that the 6S aggregate from a Φ X174 infection contains all four G products, although we cannot determine whether a single complex contains all four; both G₂ and G₃ are clearly seen (lane 11). There is also a 12S aggregate of F and G proteins⁷ and we find that it too contains all four G species (data not shown).

In addition to the presence of multiple species, there are conditions in which the G proteins show altered electrophoretic mobilities. Examination of extracts of infected cells shows that the G proteins from the *amE3* mutant have slightly greater mobilities than the G proteins of Φ X174 wild type or of nonsense mutants in genes *A*, *B*, *C*, *D*, *F* or *H*. This can be seen here (Fig. 2, lane 4) only for G₁, but in fluorograms exposed to reveal preferentially the positions of the G bands the altered mobilities were repeatedly observed (data not shown). (We assume that *amE3* contains a cryptic mutation in gene *G* that had not been previously noticed.) The altered mobility of the G proteins from *amE3* infection serves as further evidence that the proteins labelled G derive from the same genetic region.

The multiple wild-type and *amE3* G proteins do not seem to result from protein modification of a progenitor. Pulse and chase experiments carried out to examine this possibility indicated that the relative amount of label in the G bands does not change with time for a wild-type or *amE3* infection.

The four G bands could be explained by four initiation sites or by two initiation and two termination sites. If internal initiation occurs at ATG, then there are only two possible sites, nucleotides 2,570 and 2,819. The latter is unlikely because the molecular weight of the polypeptide would be reduced by about 80%, which is not observed. Therefore, we favour internal initiation at site 2,570. Readthrough could explain two additional bands. That we have only succeeded in observing one G band of phage G4 (I.T. and B. Steffen, unpublished observations) supports the readthrough; gene *G* of G4 terminates in TAA rather than TGA (G. N. Godson, personal communication) and would not be expected to read through. To explain all four G bands in Φ X174 by four initiator sites would require the assumption that two other initiating codons such as GTG or TTG are used.

The products of genes *C* and *K*

One low molecular-weight band on an SDS-polyacrylamide gel was shown previously to be the product of gene *C* (ref. 10). Figure 2 shows that not just one, but two protein species are absent when extracts are prepared from nonsuppressing cells infected with either S13 *ocC286* (lanes 12, 13) or Φ X174 *ocC6* (lanes 14, 15) and a new band, C', is seen, which is discussed later. The elimination of two bands was initially interpreted as indicating multiple products for gene *C* as well¹¹. The complete sequence in the reading frame prematurely terminated by the *ocC6* mutation at base 196 is assumed to extend from the ATG at nucleotide 133 to the TGA termination codon at nucleotide 391 and should code for a product with a calculated molecular weight of 10,069 (ref. 1). Although anomalies in protein migration are accentuated in the low molecular-weight region of gels, the observed size of the larger of the two bands, 9,600 daltons (relative to a set of CNBr-fragments of myoglobin), agrees with the calculated value, and this band is designated C.

What is the faster migrating protein species eliminated by *ocC6*? This may prove to be identical with protein K that

initiates at base 51 in gene *A*, in a reading frame different from the frames used for genes *A* or *B*, and ends with base 221 inside gene *C* (ref. 4). We have called this band K for the following reasons: (1) its high leucine to lysine incorporation ratio, 3.2 ± 0.5 , agrees with the ratio of 2.8 calculated from the sequence; (2) its mobility implies a polypeptide of molecular weight about 8,000, which is consistent with the predicted size of 6,400; (3) the band is absent from extracts of cells infected with *amA62* (data not shown). In the last case, *amA62* was derived from *am6* (ref. 3), which contains a C→T mutation at nucleotide 57 that would cause a Ser→Leu missense mutation in gene *A* (ref. 8) but an Arg→*opal* nonsense mutation in the reading frame of gene *K*.

The new band, C', seen in Fig. 2 (lanes 13, 15), resulting from an *ocC* infection, is apparently not a fragment of C terminated at the *ochre* codon but rather the result of reinitiation of translation downstream from the *ocC* site. We have determined that in Φ X174 the incorporation ratio of leucine: lysine in the C' band is greater than 7:1. If ribosomes reinitiated translation at nucleotide 230 (ATG) they would continue until nucleotide 329 (TAA), producing a protein of molecular weight 3,800 with the distinctive composition of seven leucines and no lysine. Nothing like this unusual ratio can be found in any of the other reading frames within the *C* region. (Reinitiation could conceivably occur at nucleotide 218 (CTG) instead of 230 and still be consistent with our amino acid ratio. Note that 218 and 230 are in the same reading frame.) The reinitiation phenomenon has already been demonstrated for the *rIIb* region of phage T4 (ref. 12) and the *lacZ* (ref. 13) and *i* genes^{14,15}. What makes the present case unusual is that the reinitiation, which produces a stable polypeptide, occurs in a new reading frame.

Multiple products for phages S13 and G4

S13 shows multiple A, A* and G bands analogous to those of Φ X174. G4 also shows the doublets of A and A* (J. French, personal communication) but, as previously mentioned, we have seen only one band of G. In G4 additional multiple bands have been observed, a pair for F and a pair for H; an *amF* mutant eliminates both F bands, some *amH* mutants eliminate the higher molecular-weight H band and others eliminate both (I.T. and B. Steffen, unpublished data). As neither gene *F* nor *H* terminates in TGA (G. N. Godson, personal communication) these seem to be genes having at least two initiation sites each.

A substitute for heterozygosity

Little is known about the function of the variant proteins or whether they are all essential. For the G proteins all the species seem to be functional, as they are found associated with mature phage particles in proportions roughly equal to what is synthesised in infected cells during short pulses. These small viruses have acquired variants of several of their gene products by modifying the lengths of their N-terminal regions. This has been achieved through the use of multiple initiation sites (most probably four in gene *A*, at least two in *G*, and for phage G4, two in each of genes *F* and *H*). Although a full understanding of the functions of these variant proteins is yet to be achieved, it is likely that they provide the organisms with selective advantages. It seems quite reasonable that similar advantages could be achieved in some cases by varying the lengths of the C-terminal regions if the means were available; the TGA (*opal*) terminating codon provides the means.

Of the three known chain-terminating codons, TGA is generally the most leaky¹⁶⁻²⁰. For phage S13, conditionally lethal *opal* nonsense mutants are site specific in their degree of leakiness and some Str^r hosts effectively eliminate the leakiness²¹. Readthrough is known for the natural UGA terminator of gene 3 of the RNA phage Q β (refs 22-24) and is essential for the formation of viable particles²⁵. Growth of this phage can be inhibited in a Str^r host²⁶. Readthrough *in vitro* has been shown for the product of the *O* gene of phage λ , which

terminates in TGA, and the readthrough can be eliminated by using ribosomes from Str^r cells to translate the message²⁷.

We suggest that the frequent use of the TGA terminating codon in the Φ X174 genome is designed to provide a variety of terminal sequences. This potential for variety also suggests a rationale for the existence in nature of potent *amber*-suppressing bacterial strains (Sup_{UAG}). Their existence has been a puzzle because an *amber* suppressor would seem to complicate the problem of terminating translation. But suppression of *amber* codons would have a benefit similar to that proposed for readthrough of the *opal* codons.

The capacity to initiate translation from more than one site, to read through a terminal TGA codon, or to modify a progenitor protein differentially might be a frugal way to mimic aspects of heterozygosity and could confer an advantage by creating for a single gene a heterogeneity that would aid in adaptation to variable growth conditions. In some cases each of the multiple products may even serve a substantially different function.

Φ X174 is a striking example of efficiency in deriving the most protein from the least DNA. Figure 1 shows the overlap of the nucleotide sequences of the structural genes. The Φ X174 genome of 5,375 nucleotides would code for about 200,000 daltons of protein if it used an average coding ratio of three nucleotides per amino acid. The sum of the molecular weights of the previously recognised Φ X174 proteins^{5,10,28-31} plus the protein species reported here is about 400,000.

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letters to nature

M82: the exploding galaxy?

It is nearly 20 yr since M82 was accepted as the archetypal exploding galaxy. Its most spectacular feature is the system of luminous H α filaments radiating from the core of the galaxy in the direction of the minor axis (ref. 1 and K.T. and D.J.A. in preparation), and the discovery of a velocity gradient within these filaments led to the conclusion that ionised material had been ejected from the nucleus at approximately 10^3 km s⁻¹ by a cataclysmic explosive event some 10^6 yr ago². A later and more extensive spectroscopic study refined and generally confirmed the explosion hypothesis³. It had been assumed that the emission was intrinsic to the filaments, but additional radio and optical data cast doubt on this assumption and hence the whole concept of an explosive event. The well ordered symmetrical pattern exhibited by the broad band polarisation measurements⁴ was interpreted as a consequence of reflection of the light from the main body of the galaxy by an extensive halo of dust particles⁵. Furthermore, the emission lines were also found to be polarised identically to the continuum^{6,7}; this strongly implied that they were also seen in reflection and, therefore, the observed velocity differences had to be reinterpreted in terms of motions of the scattering particles. An explosion model then becomes most unlikely, because then the dust would scatter exclusively redshifted light, whereas both blue and redshifts are observed⁸. M82 is enveloped in a giant HI cloud which engulfs the whole of the M81 group^{9,10} and Solinger *et al.*¹¹ therefore suggested that M82 is merely an

interloper in the group, moving through this dusty HI cloud in such a way that its drift velocity accounts for the observed Doppler field seen in reflection. We draw attention here to some spectacular new spectroscopic data, which we believe to be irreconcilable with these more recent scattering models and which naturally lead us to resurrect an expulsion hypothesis.

The observations presented here were obtained on the night of 28 March 1978, using the image tube spectrograph at the cassegrain focus of the Isaac Newton telescope at Herstmonceux. The detector was a nitrogen-hypersensitised IIIa-J plate behind an EMI image tube. Spectra in the vicinity of H α , at a reciprocal dispersion of 42 Å mm⁻¹ were obtained at two locations within the filamentary structure to the south of the galaxy, as part of a more detailed study, which will be reported elsewhere (D.J.A. *et al.* in preparation).

The slit length, orientation and positions are shown in Fig. 1, superimposed on a 17 Å band-pass H α electronograph obtained with the 40 mm McMullan camera at the 1 m telescope of the Wise Observatory, Israel. The spectra themselves are shown in Fig. 2. Clearly visible are the lines of H α and [NII] and it is immediately apparent that the lines are split, and more remarkably, that each forms a complete hollow ellipse. To our knowledge this line structure is unique in extragalactic studies and is reminiscent of the line profiles observed in the expanding shell of the Crab Nebula¹².

In both positions the line splitting is visible over approximately 60 arcs, but the velocity splitting between the components is slightly different. In position A the maximum velocity separation is 270 ± 5 km s⁻¹ but in position B it is only

$240 \pm 5 \text{ km s}^{-1}$. The single component regions of the lines show mean velocities, relative to the observed recession velocity of M82, of -140 and $-150 \pm 10 \text{ km s}^{-1}$, respectively. Taking into account the measuring errors, these latter velocities are not statistically different, whereas the increase of the splitting with distance from the core of the galaxy is significant, even though the base line is short.

Although a detailed model of the filamentary structure must await analysis of the complete survey, even a preliminary consideration of the data from these two positions shows that they are incompatible with the model advocated by Solinger *et al.*¹², and any attempt to explain them in terms of models invoking scattering alone seems to require such contrived geometries as to be hardly credible. We should also point out that the observed line widths in the filaments are considerably narrower than the lines observed in the main body of the galaxy. Such line width changes cannot be very easily accommodated by scattering models.

The natural explanation for our observations is one of expansion, the simplest geometrical interpretation compatible with our data being that the filaments form an expanding cone. The pronounced asymmetry of the velocity ellipse in position A

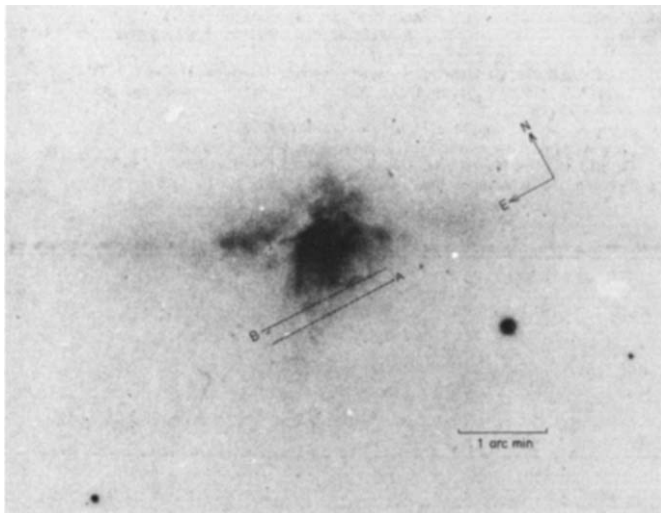


Fig. 1 A 3-h electronograph of M82, taken in the light of H α on G5 nuclear emulsion. The slit positions discussed in the text are marked as A and B.

implies that the axis of the cone is tilted towards us. Of greater significance is that we see no emission in the core of the lines between the two velocity components, implying that the emitting material is confined to the surface of the cone. From such considerations we may derive an approximate expulsion velocity of 400 km s^{-1} for the filamentary material. It is important to realise that the hollow cone geometry is fundamentally different from the original solid cone geometry of Lynds and Sandage, and we note that the axial symmetry of expulsion may be closely related to the observed phenomena in QSOs and double radio galaxies.

We should again emphasise the remarkable nature of these results, but point out that there are some obvious problems with our interpretation. Foremost amongst these is the explanation of the equivalence of the observed emission and continuum polarisations, although this has only been observed in one position to the south of the galaxy where the lines are not seen to be split. This problem may be reconciled with our observations if the observed filamentary emission is intrinsic and unpolarised but is superimposed on a weaker diffuse and highly polarised scattered component. Additional emission line polarisation measurements will be needed to develop a coherent model of this enigmatic galaxy.

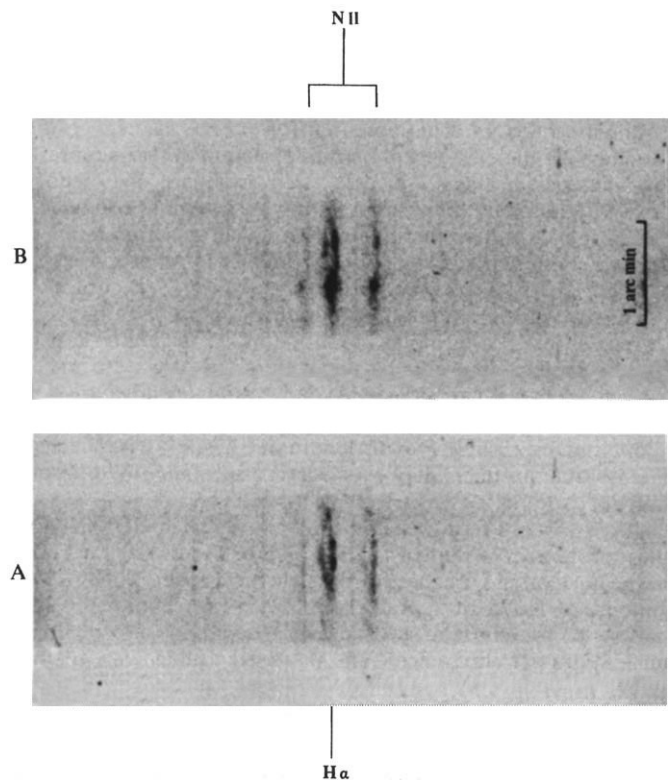


Fig. 2 Long slit spectra from the regions A and B at a dispersion of $42 \text{ \AA mm}^{-1}$. The spectral lines are as identified; wavelength increases to the right and east is to the top.

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Neutron beams in active galactic nuclei

ELECTRODYNAMIC processes may be responsible for both the creation and acceleration of charged particles near condensed objects. For pulsars^{1–3}, the strong magnetic fields associated with the spinning neutron star generate substantial electric fields which may, in turn, create electron–positron pairs. In some models³ the escaping ultrarelativistic positrons

create further pairs by curvature radiation, and can explain many features of the pulsar radiation. Models for double radio sources have also been proposed, where a magnetised rotator, say a spinar⁴ or the magnetised accretion disk around a supermassive black hole⁵⁻⁷, accelerates^{5,6} and possibly creates⁷ relativistic charged particles. Theories invoking an aligned rotator also provide a natural way of creating well collimated supersonic beams at very small distances from the powerhouse, obviating the need for hydrodynamic confinement by flattened gas clouds^{7,8}. We propose here a variation of this idea in which high energy beams of neutrons can be used to transport energy to relatively large distances from an active central powerhouse assuming that electromagnetic acceleration mechanisms are important. Decay back into charged particles allows radiation to recommence further away from such a generator than in other models.

Convenient assumptions relating to the structure of the magnetosphere outside the light cylinder³ or light surface⁶ break down where inertial forces dominate the electromagnetic ones. Even when inertial forces are unimportant, the magnetic field lines beyond the light surface have never been shown to be well collimated. An excessive amount of radiation, and thus energy loss close to the source, seems to trouble some models^{3,5}, but may be avoided if Compton losses dominate and if a large scale Poynting flux can be established⁷. Kelvin-Helmholtz and firehose instabilities could be highly disruptive, and counterflow by oppositely charged particles can cause at least partial shorting out of the beams⁵. The very high energy ($E \geq 10^{19}$ eV) beam particles of some models⁵ have such large gyroradii that it is difficult to understand how their energy is converted into radio-emitting particles inside the galaxy.

Most of these problems can be avoided if the bulk of the energy is carried away from the generator in the form of high energy neutrons. If such neutral beams can be formed from collimated charged beams rather close to the source, they will remain well collimated, not being affected by the divergence of magnetic field lines. They would not radiate as they move outwards and hence they provide a very efficient energy-momentum transport mechanism. Various plasma instabilities also disappear, and the hydrodynamic ones need be considered only far away from the source, after the neutrons decay.

Here we point out that even a very small counterflow of photons towards the surface of the rotator may convert most of a proton beam into a neutron beam by photomeson production. Outgoing photons may serve as well, if the momentum anisotropy $p_{\perp}/p_{\parallel}$ of the beam particles, or photons, is ≥ 0.1 , depending on the respective energies of the proton and photons. To fix these ideas, we choose the following parameters, consistent with a giant rotator model of active galactic nuclei: $R = 3 \times 10^{14}$ cm, is the size of the rotator; $r = 10^{13}$ cm, is the radius of the beam near the polar cap; $L = 10^{46}$ erg s⁻¹ is the total beam luminosity; $L_R (\ll L) = 10^{43}$ erg s⁻¹, is the luminosity in the counterstreaming photons, and $E_{ph} = 1$ keV, is their characteristic energy. The density of the counterstreaming photons is then $\sim 10^{15}$ cm⁻³. Ultrarelativistic ($\gamma \geq 10^5$) protons are converted to neutrons by photopion production over a typical length of 10^{13} cm ($\ll R$).

Relativistic neutrons produced with an energy $E_n = \gamma_n m_n c^2$ will travel an average distance of $2.75 \times 10^{13} \gamma_n$ cm before decaying into protons and electrons. For $\gamma_n = 10^5$ this amounts to ~ 1 pc, and assuming such energies can be attained, energy is readily transported to safe distances from the strong magnetic and radiation fields near the source. Synchrotron radiation would be generated only at this distance, which fits VLBI observations of elongated small scale structure^{11,12}.

The reconstituted, but still collimated, beams of electrons and protons continue moving outwards, no doubt entraining much interstellar matter, and pour into giant radio source lobes. Our picture then starts to look like previous beam models^{8,9,13}, with most of the energy carried in the bulk motion of the charged particles. Little radiation is to be expected until the beam is halted due to the interaction with external

confining gas, when the particles are randomised and accelerated¹⁴. We are working on the application of this mechanism in models of double radio sources and QSOs.

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Correlation between supercluster membership and richness for Abell clusters

THE Abell catalogue¹ contains 2,712 clusters, of which 1,682 form a homogeneous statistical sample, with clusters of richness $R = 1$ to 5. Murray *et al.*² have analysed the entire sample of 2,712 Abell clusters with a set of uniform criteria which identify 21 superclusters. Their criteria are: (1) that clusters which belong to the supercluster are approximately equidistant from us; M_{10} , the magnitude of the tenth brightest cluster galaxy, is allowed to range over ≤ 0.3 mag for the member clusters; (2) six or more such clusters are contained in a circle of radius θ centred on one of the clusters; and (3) $\theta \sim 1.5^\circ$ for a distance class-6 cluster and scales inversely with distance according to M_{10} . These criteria identify high-multiplicity ($n \geq 6$) superclusters, rather than groups which are predominantly binary and triplet clusters. While $\sim 40\%$ of nearby Abell clusters are in low-multiplicity ($n = 2, 3$) groups^{3,4}, only $\sim 6\%$ of all Abell clusters are contained in the superclusters of high multiplicity. This letter shows (1) that clusters of galaxies which are located in superclusters (second-order clusters of ~ 30 Mpc radius) are systematically richer than those in the 'field', and (2) that the expected higher X-ray luminosities of these richer clusters may, therefore, account for much, if not all, of the possibly large X-ray luminosities from superclusters.

For the purposes of a richness analysis of the supercluster sample one should consider only clusters belonging to Abell's statistical sample of 1,682 objects; in this instance, application of the Murray *et al.* criteria yields 14 superclusters (their supercluster nos 3, 4, 6, 14, 16, 18 and 19 are excluded).

Table 1 lists the number of clusters of each richness class in the Abell statistical sample and in the sample of 14 superclusters (which contains 102 clusters). The percentages in each class and the mean richness for each sample are also given. Table 1 also shows that clusters in the supercluster sample are richer than average; the percentage of clusters with $R \geq 2$ is 1.5 times as large in the supercluster sample as in the Abell sample.

Table 1 Richness of clusters in superclusters

<i>R</i>	Abell		14 Superclusters	
	No.	%	No.	%
1	1,224	73	61	60
2	383	23	35	34
3	68	4	5	5
4	6	0	1	1
5	1	0	0	0
≥ 2	458	27	41	40
$\langle R \rangle$	1.32		1.47	

The probability of this occurrence being produced by a random draw from the Abell sample is small. For simplicity, consider two categories, $R = 1$ and $R \geq 2$. The probability of a random sample of 102 clusters having 41 or more members with $R \geq 2$ is found to be 3.0×10^{-3} . I conclude that the supercluster sample is richer with high probability. Rood³ found no correlation between richness and (low-multiplicity) group membership for nearby clusters.

There is also a weak correlation in favour of Bautz-Morgan⁵ clusters of type III appearing in superclusters. Table 2 gives the distribution of Bautz-Morgan types, taken from Leir and van den Bergh⁶, for the Abell and supercluster samples. The probability of drawing 63 or more type III clusters out of a random sample of 99 objects is 9.7×10^{-2} . Thus, there is a marginally significant preference for the clusters in superclusters to belong to the latest, most irregular, Bautz-Morgan type. Rood³ has found a similar preference for type III clusters among nearby low-multiplicity groups. He also found a preference for types I and I-II; this preference does not occur in the present high-multiplicity sample.

Table 2 Bautz-Morgan classifications for clusters in superclusters

B-M classification	Abell		14 Superclusters	
	No.	%	No.	%
I	58	3	2	2
I-II	64	4	1	1
II	233	14	12	12
II-III	374	22	22	22
III	954	57	62	63

Rood-Sastry⁷ classifications are not available for the distant clusters which are in the supercluster sample above. For the nearby groups (which typically contain only two or three clusters) listed by Rood and Sastry there is no apparent correlation between group membership and cluster Rood-Sastry classification.

Perrenod⁸ has argued that gravitational instability in the supercluster environments should lead to more massive clusters that are stronger X-ray sources. As cluster richness is based on galaxy counts and is thus a rough measure of cluster mass⁹, the supercluster-richness correlation presented here supports the gravitational instability arguments.

Superclusters may be strong X-ray emitters. The Uhuru group² has observed three candidate sources of average $L_x \geq 3 \times 10^{45} \text{ erg s}^{-1}$. However, the OSO 8 upper limit¹⁰ for 4U0134-11 is $L_x < 1.3 \times 10^{45} \text{ erg s}^{-1}$, and the Ariel 5 survey has not detected at least two of the three candidate sources¹¹. Thus the sources may not be real, or they may be variable, which would rule out cluster or supercluster identifications. If the individual clusters in superclusters have X-ray luminosities equal to their 'field' counterparts, one expects a total $L_x \sim 1 \times 10^{45} \text{ erg s}^{-1}$, on average. If the Uhuru supercluster sources

are real, and the identifications correct, then either clusters in superclusters are brighter X-ray sources or there is X-ray emission from hot or relativistic gas which fills much of the supercluster volume outside of the clusters.

The richness correlation shown above is probably responsible, at least in part, for the possibly high supercluster X-ray luminosities. A correlation between cluster richness class and X-ray luminosity seems to exist. In the compilation of Jones and Forman (unpublished), the average luminosity of those $R = 1$ clusters which are detected is $L = 6.4 \times 10^{44} \text{ erg s}^{-1}$, and for the detected clusters of $R = 2$ the average luminosity is nearly twice as great, $L = 11.4 \times 10^{44} \text{ erg s}^{-1}$. As a larger fraction of the nearby $R = 2$ clusters have been detected as X-ray sources, the relative enhancement in luminosity is probably > 2 .

Unfortunately, two of the three X-ray supercluster candidates of Murray *et al.* do not belong to the homogeneous sample of 14 superclusters used to construct Table 1. The one supercluster which does, however, 4U0134-11, is quite rich. It contains one $R = 3$ cluster, two $R = 2$ clusters and three $R = 1$ clusters, plus an $R = 0$ cluster. The probability of drawing three or more $R \geq 2$ clusters in six attempts from the Abell homogeneous statistical sample (which excludes $R = 0$) is 0.22.

Schwartz¹² has evaluated the X-ray luminosity function for nearby Abell clusters and finds an average X-ray luminosity $L_{44} = 2.0$ where L_{44} is in units of $10^{44} \text{ erg s}^{-1}$. If $R \geq 2$ clusters are $\geq 11.4/6.4 = 1.8$ times as bright as $R = 1$ clusters, then the average luminosity for $R = 1$ clusters is $L_{44} \leq 1.6$ and for $R \geq 2$ clusters, $L_{44} \geq 3.0$, using the statistics from the Abell sample for the relative number of $R = 1$ and $R \geq 2$ clusters. Thus, the expected luminosity from 4U0134-11 is $L_{44} \geq 3 \times 3.0 + 3 \times 1.6 = 13.8$. The observed value from Uhuru (using the PST intensity which is statistically more reliable) is $L_{44} = 20.0$ and the OSO 8 upper limit is $L_{44} < 13.0$. Thus, for this supercluster, the member clusters may be sufficiently luminous to explain all of the X-ray emission, from the supercluster.

Applying similar arguments to the other two supercluster X-ray sources, one finds expected luminosities due to superposition of individual clusters of $L_{44} \geq 12.2$ and $L_{44} \geq 8.0$, for 4U0443-09 and 4U1456+22, compared with Uhuru luminosities of $L_{44} = 40.0$ and $L_{44} = 36.0$, respectively.

The three superclusters which Uhuru may have detected should represent the bright end of the supercluster X-ray luminosity function and thus using average cluster luminosities for a given richness class is probably conservative. It seems, therefore, that a significant part, but perhaps not all, of the apparently high X-ray luminosity of superclusters is explained by the greater richness and larger mass of their constituent clusters.

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New light on sunspot darkness and the solar cycle

NUMEROUS attempts have been made to find the physical mechanism responsible for the sunspot cycle. Present theoretical discussions of the basic mechanism of solar activity are usually based on a dynamo theory with a reversing magnetic field¹, but objections and alternative interpretations^{1,2} have recently been advanced. Our understanding of the sunspot cycle seems to be in a stage where new observational insight may be valuable. We report here a variation in the infrared umbra intensity of large sunspots with the phase in the solar cycle.

Differences in the intensity of sunspot umbrae have been difficult to find from measurements in the visual because of the uncertainties in the observations due to the large amount of scattered light in this wavelength region. Real and significant intensity differences have been detected, however, by Ekmann and Maltby³ from broad-band pinhole photometer observations in six wavelength regions in the visual and the infrared. Using the same type of equipment we have extended these observations to 10 wavelength regions between 0.387 and 2.4 μm . Here we consider large sunspots observed during good seeing conditions. Although this limits the number of observed sunspots it means that reliable intensities are obtained. We restrict the discussion to the results obtained in the wavelength region centred on 1.67 μm , where the intensity differences between sunspots are apparent³.

Table 1 gives the dates, sizes and heliographic latitudes, as well as umbra and penumbra intensities at 1.67 μm for all large sunspots observed in the period 1968–78. The observed intensities have been carefully corrected for stray light in the Earth's atmosphere and the instrument⁴. The intensity is only weakly dependent on other sunspot parameters³, and in particular, it does not show any apparent variation with size (see Table 1) for large sunspots.

In searching for a possible link between the physical properties of sunspots and the sunspot cycle we have plotted in Fig. 1 the relative umbra intensity against the time interval between

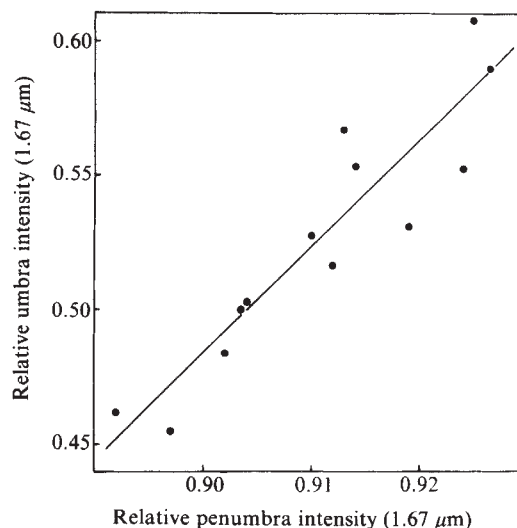


Fig. 2 The relative umbra intensity compared with the relative penumbra intensity for individual, large sunspots. Both intensities are measured relative to the photospheric intensity.

the date of sunspot observation and the epoch of minimum solar activity (that is, 1964.8 and 1976.6 for cycles 20 and 21, respectively). From Fig. 1 we find that the relative umbra intensity I_u/I at 1.67 μm may be expressed as

$$(I_u/I)_{1.67} = 0.44 + 0.15(t/t_0) \quad (1)$$

where t is the number of years from starting epoch of the solar cycle with duration t_0 . Figure 1 shows that the umbra intensity is closely correlated with t/t_0 ; the correlation coefficient is 0.92 (+0.03, -0.06) and the corresponding probability of there being no correlation is less than 1%. It may be argued that one sunspot observed twice in 1976 during the transition period from cycle 20 to 21 should be omitted. This would imply a decrease in correlation coefficient from 0.92 to 0.91.

As the heliographic latitude of sunspots varies with the phase in the sunspot cycle we have considered the possible connection between the umbra intensity and the heliographic latitude of the observed sunspots. This comparison produces a correlation coefficient of 0.6 ± 0.2 and the corresponding probability of the two quantities being uncorrelated is 5%.

Figure 2 gives the umbra intensity plotted against the corresponding penumbra intensity at 1.67 μm ; both intensities are given relative to the photospheric intensity. Figure 2 shows that there is a close connection between umbra and penumbra intensities of the same sunspot in the sense that a dark umbra is surrounded by a dark penumbra (as averaged over penumbral filaments). The data presented in Fig. 2 give a correlation coefficient of 0.92 (+0.04, -0.06) and confirm the findings of Ekmann⁵ based on a smaller sample.

The evidence presented in Fig. 1 favours a close connection between the temperature in the umbra of individual large sunspots and the phase in the sunspot cycle. On combining this with the results presented in Fig. 2, an epoch dependence seems to be present both for the umbra and the penumbra. The time variation is such that the emitted intensity from sunspots increases with epoch during the solar cycle. Taking into account the observations in the other wavelength regions, a preliminary estimate gave a 5% variation in the total flux from large sunspots during the cycle.

The change in umbra darkness with the phase in the solar cycle implies that there is a real change in the physical conditions from one sunspot to another. To have a stable sunspot the physical conditions must be such that the difference in gas pressure in the horizontal direction is compensated by the magnetic field and the velocity field. With differences in

Fig. 1 The umbra intensity as measured relative to the photospheric intensity at 1.67 μm for individual, large sunspots as functions of epoch. The number of years from the epoch of minimum solar activity is marked. All large sunspots observed during good seeing conditions in the period 1968–78 are included.

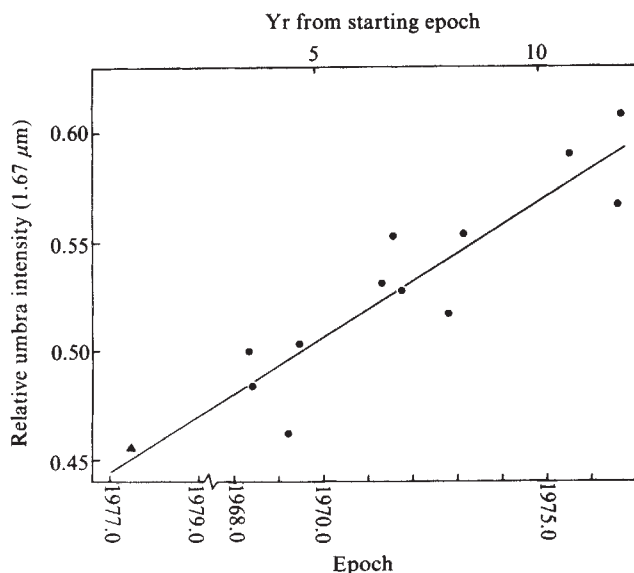


Table 1 Relative umbra and penumbra intensities at $1.67\ \mu\text{m} \pm 0.04\ \mu\text{m}$

Date	Penumbra semi-diameter	Umbra semi-diameter	Heliographic latitude	Umbra intensity	Penumbra intensity
16–17 April 1968	28", 23"	12", 8"	23°N	0.500 ± 0.011	0.904 ± 0.005
19 May 1968	29, 22	14, 8	32°S	0.484 ± 0.015	0.902 ± 0.006
21–22 March 1969	38, 28	13, 9	22°N	0.462 ± 0.011	0.892 ± 0.004
14 June 1969	37, 20	19, 8	15°S	0.503 ± 0.015	0.904 ± 0.006
18 April 1971	22, 21	10, 8	10°N	0.531 ± 0.015	0.919 ± 0.006
17 July 1971	26, 23	10, 9	6°N	0.553 ± 0.015	0.924 ± 0.006
26 September–3 October 1971	30, 22	12, 9	7°S	0.528 ± 0.015	0.910 ± 0.004
19–24 October 1972	31, 24	13, 9	14°S	0.517 ± 0.009	0.912 ± 0.004
1 March 1973	39, 25	13, 7	7°N	0.554 ± 0.015	0.914 ± 0.005
30 June 1975	25, 20	12, 10	8°N	0.590 ± 0.015	0.927 ± 0.006
5–8 August 1976	28, 22	14, 7	17°N	0.567 ± 0.007	0.913 ± 0.004
2–5 September 1976	21, 19	8, 7	16°N	0.608 ± 0.011	0.925 ± 0.006
14 June 1977	19, 16	8, 6	22°S	0.455 ± 0.010	0.897 ± 0.006

temperature stratification from one sunspot to another it is likely that the difference in depth between the observable layers in the umbra and the photosphere, called the Wilson depression, may be different for different sunspots⁶.

We have presented observations here at one wavelength; the same trend is present for the measurements in the other wavelength regions. Theoretical models should therefore predict an increase in radiative flux in large sunspots with epoch. We note that a decrease in nonradiative flux with phase in the sunspot cycle may occur if the effectiveness of the sunspot cooling mechanism decays with time within the cycle.

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Diffusion-coupled oxidation of atmospheric sulphur dioxide

INCREASING evidence of the toxicity of atmospheric sulphates, together with the upward trend in fossil fuel combustion, have made the rate of formation of atmospheric sulphates one of the most important topics in current air pollution research. Many investigators¹ have measured sulphate concentrations in plumes and air sheds, and have usually fitted their data to a linear SO₂ oxidation rate expression. The reported values thus obtained for the rate constant vary by up to two orders of magnitude¹. Moreover, the linear rate expression is inconsistent with the findings of numerous field investigators^{2–5}, whose data suggest that the oxidation occurs almost exclusively near the SO₂ source, rather than uniformly throughout a dispersing atmosphere. The analysis given here deduces that the oxidation may occur almost wholly near the source, and describes the circumstances in which this will happen. It is also shown that in these conditions the oxidation proceeds not to completion but to a fractional asymptotic limit, and that half the final sulphate yield will be obtained in an

amount of time whose limits are predictable, constant for any given dispersion pattern, and independent of the magnitude of the rate constants and other chemical parameters.

In a typical field investigation of atmospheric SO₂ to SO₄²⁻ conversion rates, the data obtained ([SO₂], [SO₄²⁻]) are fitted to expressions of the type

$$X_r(x, y, z, t) = X(x, y, z) \exp(-kt) \quad (1)$$

where $X(x, y, z)$ is given by an appropriate dispersion formula⁶, $X_r(x, y, z, t)$ is [SO₂] downwind, and a pseudo-first order oxidation with a corresponding rate constant k is postulated. The values reported¹ for k range from 0.37 to 55% h⁻¹. The discrepancies cannot at present be harmonised, because no progress has yet been made in ascertaining the functional dependencies between k and the variable factors which are thought to influence k (for example, relative humidity, temperature and the concentrations of nitrous oxides, metal oxides, hydrocarbons and other pollutants). Consequently, little insight can be derived from k besides an inference that the average rate of oxidation is faster in some conditions than in others.

Equation (1) implies that the oxidation proceeds independently of atmospheric dispersion. Second or higher order chemical reactions are coupled to atmospheric dispersion^{12–14} and thus cannot be represented by equation (1). Using equation (1) to represent atmospheric SO₂ oxidation has been rationalised by invoking laboratory studies which found the SO₂ oxidations to be first order processes. But in the atmosphere, SO₂ is transported with the mean wind and mixes with the ambient air, becoming continuously more diluted at the same time as it is being oxidised to sulphate by one or more of the paths described in Table 1. Thus, although the SO₂ chemistry within a droplet or on a particle's surface is linear, the process by which SO₂ reaches the droplet or particle is comparable to a second order reaction. Consequently, the overall process is one of chemical reaction coupled to atmospheric dispersion, and it must be modelled as such (rather than by equation (1)) even though the oxidation would be first order in a laboratory vessel of fixed volume.

Consider first an oxidation of the equation (2s) type (Table 1). The SO₂ must diffuse to the particle and react with chemisorbed O₂. But as atmospheric dispersion dilutes the SO₂ and the particulate matter, the distance between the SO₂ and the aerosol increases. Thus, the phenomenon as a whole is analogous to the chemical reaction in an expanding-volume reactor described by Benton¹⁵. Setting the expanding volume V such that:

$$V = bt^2 \quad (3)$$

and applying Benton's method to equation (2s), separating

Table 1 Rate expressions for various types of SO₂ oxidation

Type of oxidation	Rate expression	Equation no.	Definitions
Homogeneous first order, gas phase ⁷	$\frac{d[\text{SO}_4^{2-}]}{dt} = k'[\text{SO}_2]$	2'	
Heterogeneous, aerosol, surface particle ⁸	$\frac{d[A]\theta}{dt} = k_s[\text{SO}_2][A][1-\theta]$	2s	$A \equiv$ total capacity to form sulphate (mol g ⁻¹) $\theta \equiv$ fraction occupied by sulphate at time t $K_s \equiv k_s[(\text{SO}_2)_0 - (A)]$
Heterogeneous, aerosol, catalysed, buffered oxygenated solutions ⁹	$\frac{d[\text{SO}_4^{2-}]}{dt} = \frac{k_c[\text{H}_2\text{SO}_3][\text{Fe}^{+3}]}{[\text{H}^+]_{\text{buff}}}$	2c	$\beta_s \equiv [\text{H}_2\text{SO}_3][\text{SO}_2]^{-1}$ (Henry's law) $K_c \equiv k_c\beta_s[\text{Fe}^{+3}]/[\text{H}^+]_{\text{buff}}^2$
Heterogeneous, aerosol, uncatalysed, buffered oxygenated solutions ¹⁰	$\frac{d[\text{SO}_4^{2-}]}{dt} = \frac{k_u[\text{H}_2\text{SO}_3]}{[\text{H}^+]_{\text{buff}}^2}$	2u	$\beta_s \equiv [\text{H}_2\text{SO}_3][\text{SO}_2]^{-1}$ (Henry's law) $\lambda_s \equiv [1-p/p_0][\text{SO}_4^{2-}]^{-1}$ (Raoult's law) $K_u \equiv k_u\beta_s[1-p/p_0]/\lambda_s[\text{H}^+]_{\text{buff}}^2$
Homogeneous second order, gas phase ¹¹	$\frac{d[\text{SO}_4^{2-}]}{dt} = k''[\text{SO}_2]^2$	2''	

variables and integrating analytically, we obtain the following fractional yields:

$$\theta_t = \frac{(\text{SO}_2)_0(1 - E_{ts}E_{0s})}{(\text{SO}_2)_0 - (A)E_{ts}E_{0s}} \quad (4s)$$

and

$$\theta_\infty = \lim_{t \rightarrow \infty} \theta_t = \frac{(\text{SO}_2)_0(1 - E_{0s})}{(\text{SO}_2)_0 - (A)E_{0s}} \quad (5s)$$

where: b is a constant whose value depends on meteorological conditions; t_0 is the time at which $V =$ initial volume V_0 ; $E_{ts} \equiv \exp(K_c/bt)$; and $E_{0s} \equiv \exp(-K_s/bt_0)$. (Note that $(x) \equiv$ amount of x in mol and $[x] \equiv$ concentration of x in mol l⁻¹ for any substance x . Thus $[x] = (x)/V$. $(x)_t$ and $[x]_t$ are (x) and $[x]$ at time t . $(x)_0$ and $[x]_0$ are x and $[x]$ at time t_0 . In setting $V = bt^2$ we have postulated a particular case of $V = bt^n$, a general form in which dispersion patterns can be described⁶.)

As $\theta_\infty < 1$, it is appropriate to calculate the half life of the reaction (τ_s) rather than the half lives of the reactants. We obtain:

$$\frac{1}{\tau_s} = \frac{1}{t_0} + \frac{b}{K_s} \ln \frac{(\text{SO}_2)_0 - [2(A) + (\text{SO}_2)_0]E_{0s}}{(\text{SO}_2)_0 - (A)} \quad (6s)$$

Using the same approach for the other oxidations given in Table 1 (after making the substitutions indicated in the 'definitions' column), we obtain the fractional yields and half-life expressions shown in Table 2.

In a laboratory vessel of fixed volume, the heterogeneous oxidations (equations (2s), (2c) and (2u) in Table 1) are functionally identical to each other and to the first order homogeneous oxidation (equation (2')), and all proceed to completion. But on coupling the oxidation to the atmospheric dispersion we obtain the yield equations (4'), (4s), (4c) and (4u) (see Table 2), each of which is functionally different from every

Table 2 Yields and characteristic times of SO₂ oxidation

Chemistry	Fractional yields		Characteristic times	Limit of τ for		Definitions
	$(\text{SO}_4^{2-})_t/(\text{SO}_2)_0$	$(\text{SO}_4^{2-})_\infty/(\text{SO}_2)_0$		$R \rightarrow \infty$	$R \rightarrow 0$	
Homogeneous first order	$1 - \exp(-k'(t-t_0))$ (4')	1 (5')	$t_0 + \frac{\ln 2}{k'}$ (6')	t_0	∞	$R' \equiv k't_0$
Heterogeneous solution catalysed	$1 - E_{tc}E_{0c}$ (4c)	$1 - E_{0c} < 1$ (5c)	$\left[\frac{1}{t_0} + \frac{b}{K_c} \ln \left(\frac{1 + E_{0c}}{2} \right) \right]^{-1}$ (6c)	t_0	$2t_0$	$E_{tc} \equiv \exp(K_c/bt)$ $E_{0c} \equiv \exp(-K_c/bt_0)$ $R_c \equiv K_c/bt_0$
Heterogeneous solution uncatalysed	$\frac{1 - E_{tu}E_{0u}}{1 + \frac{(\text{SO}_2)_0}{(\text{SO}_4^{2-})_0} E_{tu}E_{0u}}$ (4u)	$\frac{1 - E_{0u}}{1 + \frac{(\text{SO}_2)_0}{(\text{SO}_4^{2-})_0} E_{0u}} < 1$ (5u)	$\left[\frac{1}{t_0} + \frac{b}{K_u} \ln \left(\frac{(\text{SO}_4^{2-})_0 + [2(\text{SO}_2)_0 + (\text{SO}_4^{2-})_0]E_{0u}}{2(\text{SO}_4^{2-})_0 + (\text{SO}_2)_0[1 + E_{0u}]} \right) \right]^{-1}$ (6u)	t_0	$2t_0$	$E_{tu} \equiv \exp(K_u/bt)$ $E_{0u} \equiv \exp(-K_u/bt_0)$ $R_u \equiv K_u/bt_0$
Homogeneous second order	$\frac{k''(\text{SO}_2)_0(t-t_0)}{bt_0 + k''(\text{SO}_2)_0(t-t_0)}$ (4'')	$\frac{1}{1 + bt_0/k''(\text{SO}_2)_0} < 1$ (5'')	$\frac{2 + k''(\text{SO}_2)_0/bt_0}{1 + k''(\text{SO}_2)_0/bt_0}$ (6'')	t_0	$2t_0$	$R'' \equiv k''(\text{SO}_2)_0/bt_0$

The reaction yields and the characteristic times are functionally different for every oxidation; but whereas neither the heterogeneous oxidations nor the second order homogeneous oxidation proceeds to completion and their characteristic times all have the same limits, the first order homogeneous oxidation proceeds to completion and its characteristic time is not limited.

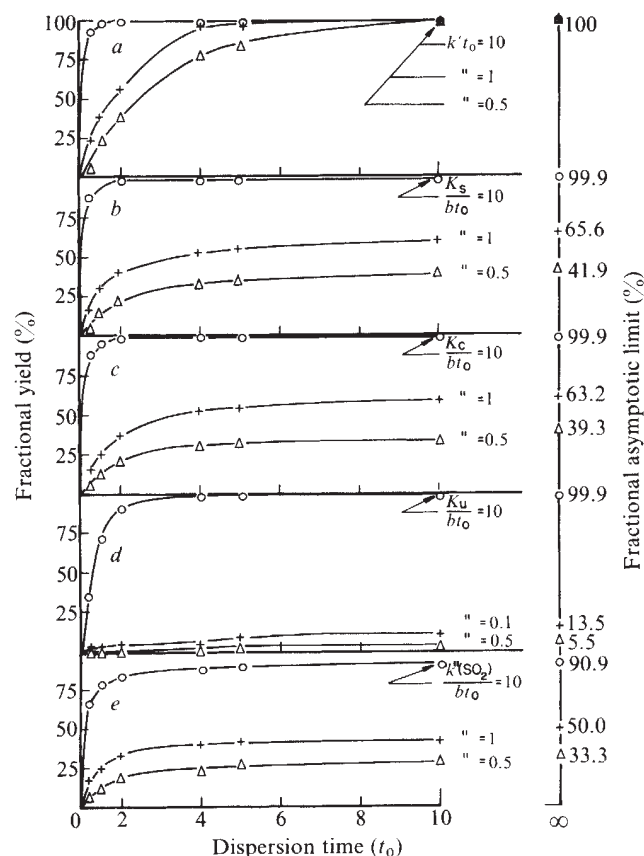
other, as are the reaction half-life expressions (6'), (6s), (6c) and (6u) (see Table 2). However, there are several notable parallels which should be discussed. All the heterogeneous oxidations proceed not to completion but to fractional asymptotic limits (equations (5s), (5c), (5u) in Table 2) as does the second order homogeneous oxidation (equation (5'')) (see Fig. 1). The first order homogeneous oxidation proceeds to completion as it does in a fixed volume (see Fig. 1)^{17,18}.

Thus, the first order homogeneous oxidation is independent of the dispersion process, whereas the heterogeneous oxidations are dependent on the dispersion process (as is the second order reaction). Moreover, for all the heterogeneous oxidations and for the second order homogeneous oxidation, the magnitudes of the fractional yields and of the characteristic times depend on the ratios of the chemical parameters (K_s , K_c , K_u , k_2 , $(SO_2)_0$) to dispersion parameters (b , t_0). It is easily shown that an increase in the ratios will cause an increase in the limit (so that if:

$$\frac{K_s}{bt_0} \rightarrow \infty, \quad \frac{K_c}{bt_0} \rightarrow \infty, \quad \frac{K_u}{bt_0} \rightarrow \infty, \quad \frac{K_2(SO_2)_0}{bt_0} \rightarrow \infty,$$

then $[(SO_4^{2-})_\infty / (SO_2)_0] \rightarrow 1$ for equations (5s), (5c), (5u) and (5'') respectively) and vice versa (when the ratios approach 0, $[(SO_4^{2-})_\infty / (SO_2)_0] \rightarrow 0$) (see Fig. 1). Similarly, it can be shown that the characteristic times (see equations (6s), (6c), (6u), (6'')) decrease with an increase in the ratios (so that when the ratios

Fig. 1 The sensitivity of the sulphate yield to changes in time for different ratios of chemistry to dispersion is shown. For heterogeneous or second order homogeneous oxidations the sulphate yield is especially sensitive to changes in time within the interval $t_0 \leq t \leq 2t_0$ and the sensitivity decreases with increasing time so that a limit of conversion is reached at about $t = 4t_0$. The first order homogeneous oxidation proceeds to completion and is independent of the dispersion process. *a*, Homogeneous first order; *b*, heterogeneous surface catalysed; *c*, heterogeneous solution catalysed; *d*, heterogeneous solution uncatalysed; *e*, homogeneous second order. In *b*, $(SO_2)_0 / (A)_0 = 10$. In *d*, $(SO_2)_0 / (SO_4^{2-})_0 = 10$.



approach ∞ , $t \rightarrow t_0$) and vice versa (when the ratios approach 0, $t \rightarrow 2t_0$).

Thus, half the final sulphate yield will be formed close to the source and in a time whose limits can be predicted. These limits are constant for any given dispersion pattern and do not depend on the magnitude of the rate constants and other chemical parameters or on whether the oxidation is heterogeneous or second order homogeneous. (The same conceptual results can be obtained for an air shed expanding in three dimensions, for which $V = bt^3$ (there $t_0 \leq \tau \leq \sqrt{2} t_0$) and, indeed, for any expanding atmosphere whose rate of expansion is higher than first order, for all dispersions $V = bt^n$ for which $n > 1$. Where $n \leq 1$ (that is, the reactions proceed to completion) but their yields and characteristic times depend on b and t_0 .) The homogeneous first order reaction is independent of dispersion and always proceeds to completion.

These results are consistent with the findings of recent field investigations²⁻⁵ of SO_2 oxidation. For example, Smith and Jeffrey⁴ state that their results "seem to imply that possibly most of the conversion takes place very early on near the source region." Dana *et al.*³ state that their observed sulphate concentrations "indicate a significant rapid reaction to sulphate near the stack" and that "the in-plume SO_2 oxidation process proceeds very rapidly as the plume leaves the source and attenuates as distance downwind increases, leaving higher amounts of SO_2 at larger distances than would be predicted on the basis of linear kinetics in conjunction with the given rate constants." Forrest and Newman² state that "the oxidation occurs during the early history of the plume with virtually no further conversion taking place downwind" and that the oxidation "eventually reaches an asymptotic limit of SO_2 conversion."

The present analysis does not take into account dispersion-coupled oxidations which are limited by diffusion of ambient gases (such as O_3 and NH_3)¹⁶. The mechanisms of such processes, which are of greater mathematical complexity, warrant investigation.

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Radionuclide adsorption by manganese oxides and implications for radioactive waste disposal

COBALT-60 and certain transuranics such as ^{244}Cm , ^{241}Am and ^{238}Pu are migrating in trace amounts from original intermediate-level waste disposal pits and trenches in the radioactive waste burial grounds of Oak Ridge National Laboratory (ORNL) in Tennessee¹. These radionuclides are transported in

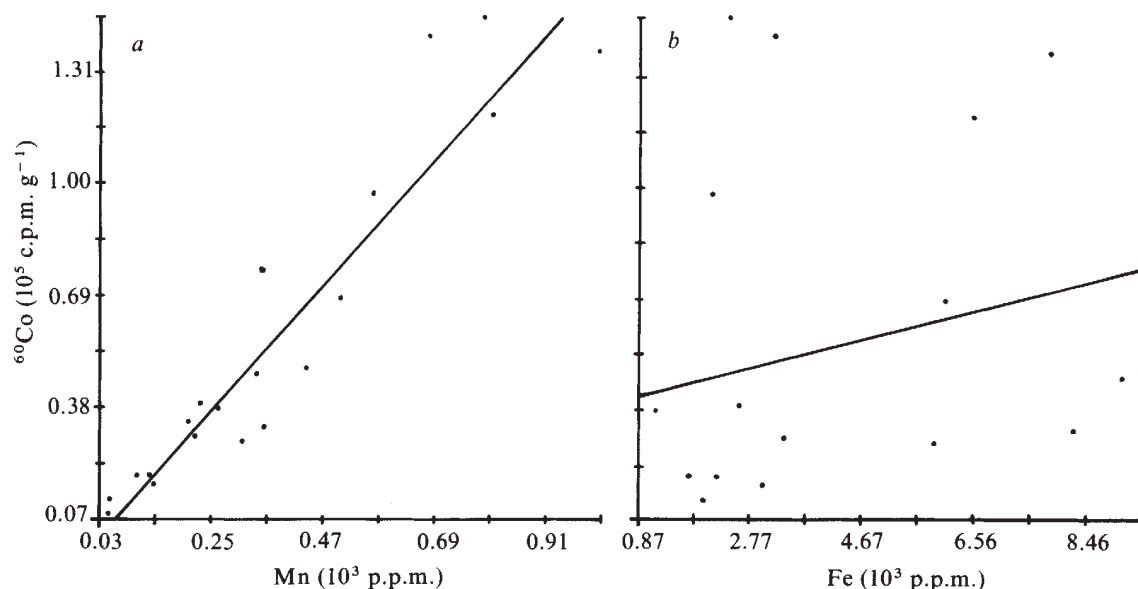


Fig. 1 Correlation coefficients, where N = number analysed; *a*, for total manganese ($R = 0.9535$, $N = 20$); and *b*, total iron ($R = 0.2696$, $N = 20$) oxide plotted against total ^{60}Co concentrations in size-fractionated soil samples from seep approximately 100 yd east of trench 7 in ORNL disposal area.

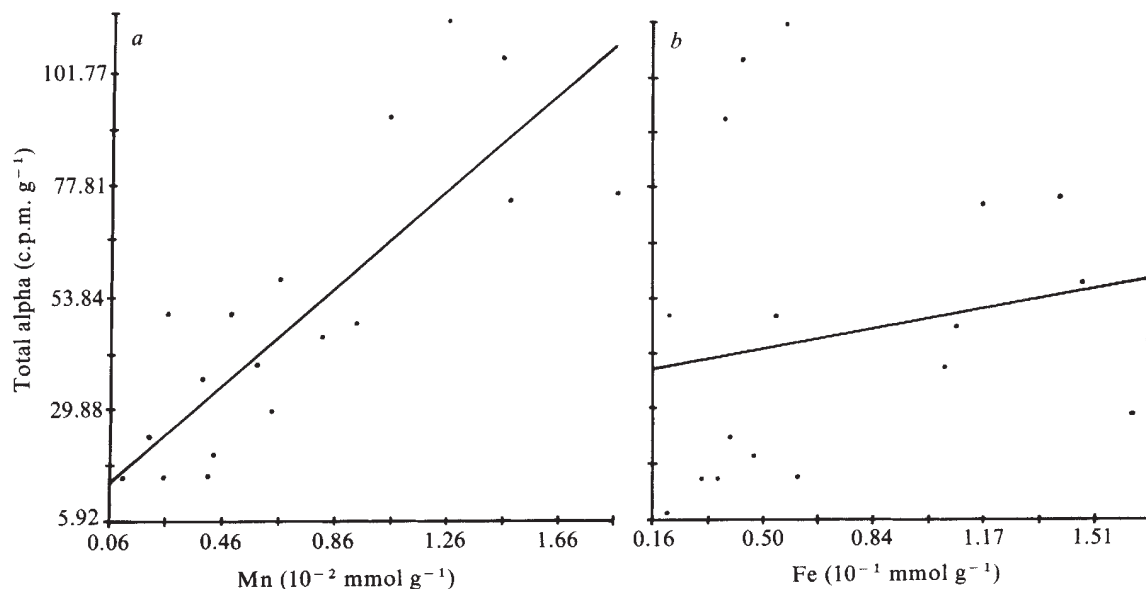
trace amounts from the trench 7 area and are to some extent adsorbed by soil and shale along the route of migration. We show here that the adsorbed radionuclides, which are very strongly bound in the soil, are being retained primarily by manganese oxides in the soils. Our data illustrate the importance of the manganese oxide component of soils and sediments in general in controlling ^{60}Co and actinide mobility. Certain implications of these results for the field of radioactive waste management will also be discussed.

To differentiate between clays and particle coatings as ^{60}Co and actinide adsorbents, soil from a seep approximately 100 yd east of trench 7 was size-fractionated, and the radionuclide contents of the different size fractions were analysed². If clays are the primary adsorbents, the 0.2–2- μm fractions should contain essentially all of the radionuclide contents. If the

adsorbents are particle coatings such as iron and manganese oxides, ^{60}Co and the actinides should be observed in all size fractions. However, the finer fractions would possess higher radionuclide concentrations because of their larger total particle surface area and consequently greater particle coating contents relative to the coarser fractions. Size-fraction distributions indicate the presence of ^{60}Co , ^{244}Cm and ^{241}Am in all fractions². Radionuclide concentrations decrease in the order: clay > very fine silt > medium silt > coarse silt > sand, suggesting adsorption by particle coatings rather than clays.

Analyses were carried out to differentiate the roles of various particle coatings in radionuclide adsorption. Total iron oxyhydroxide (mostly amorphous with some goethite) and manganese oxide (entirely amorphous) contents of the size-fractionated soils were analysed using wet chemical

Fig. 2 Correlation coefficients; *a*, for total manganese ($R = 0.8398$, $N = 20$); and *b*, total iron ($R = 0.2139$; $N = 20$) oxide plotted against total alpha contents of size-fractionated soils from seep east of trench 7. Alpha-emitting radionuclides are comprised of approximately 60% ^{244}Cm , 35% ^{241}Am , with traces of ^{238}Pu , ^{224}Ra and ^{228}Th .



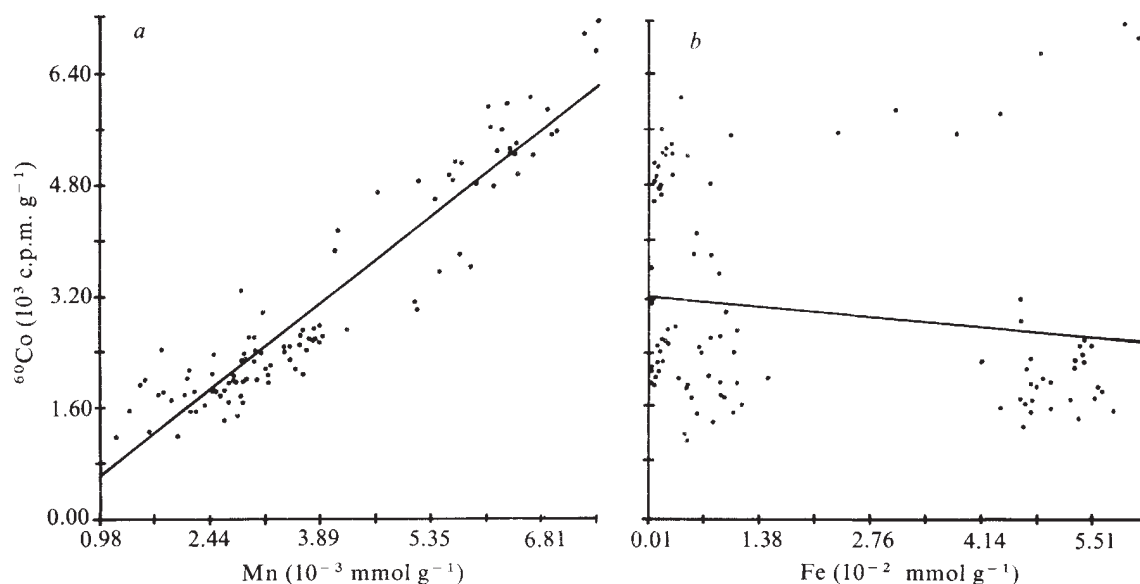


Fig. 3 Correlation coefficients for ^{60}Co plotted against selectively extracted *a*, manganese ($R = 0.9425$, $N = 107$) and *b*, iron ($R = -0.1181$, $N = 107$) oxides from trench 7 seep soil using hydroxylamine hydrochloride, ammonium oxalate and to a lesser extent, potassium pyrophosphate.

techniques^{2,3}. Statistical correlation analyses were then carried out on manganese and iron oxide values plotted against soil radionuclide contents. Strong positive correlations between manganese oxide and radionuclide concentrations were observed, with correlation coefficients, R , of $+0.95$ and $+0.84$ for manganese oxide against ^{60}Co and total alpha, respectively (Figs 1*a* and 2*a*). Iron oxyhydroxide and radionuclide contents are poorly correlated, with $R = +0.27$ and $+0.21$ for ^{60}Co and total alpha, respectively (Figs 1*b* and 2*b*). Similar analyses involving organic carbon and radionuclide concentrations demonstrate no or negative correlations.

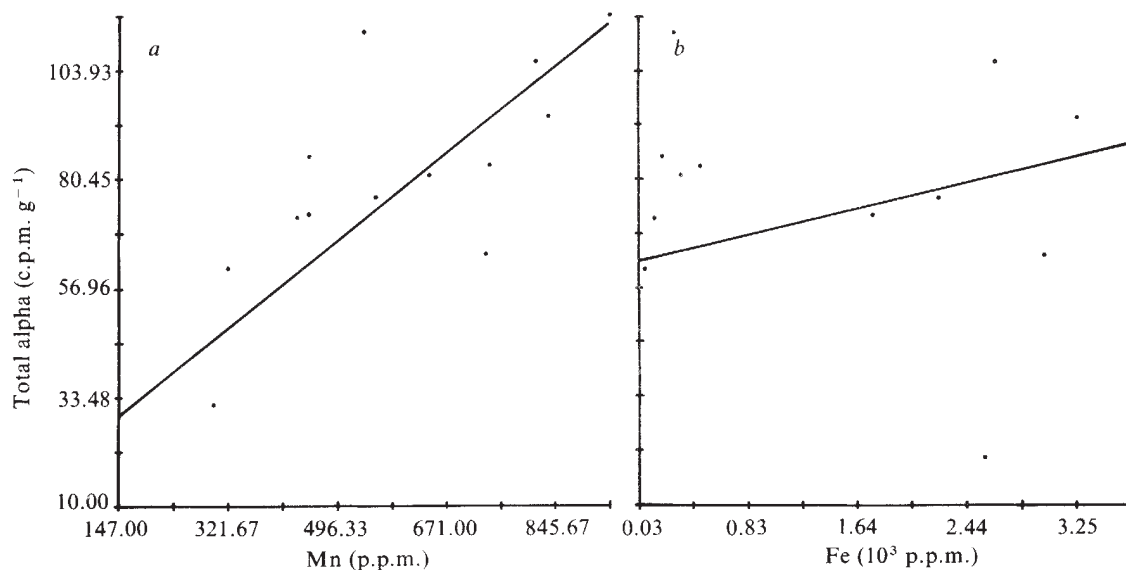
Further correlations based on repeated selective hydroxylamine hydrochloride and ammonium oxalate extractions of Fe and Mn in different soils from the trench 7 area yielded similar results: ^{60}Co and the actinides present are

predominantly associated with and strongly retained by manganese oxides (typical results in Figs 3 and 4). Even though iron oxyhydroxide and insoluble organic carbon concentrations range from 2 to 20 times greater than manganese oxide contents in the soils examined², a positive correlation between ^{60}Co , actinides and manganese oxides is clearly demonstrated.

The manganese oxides have long been recognised as important scavengers of trace metals in the natural environment⁴⁻⁶. For example, in seawater, concentrations of metals such as Co, Cu, Ni, Ag, Au and Pb are undersaturated by many orders of magnitude, owing to the adsorptive properties of ferromanganese deposits⁷.

The important role of manganese oxides in trace metal adsorption can be attributed to its unique surface and colloidal properties. The surface areas of ferromanganese nodules and

Fig. 4 Correlation coefficients for total alpha plotted against selectively extracted *a*, manganese ($R = 0.7960$, $N = 15$) and *b*, iron ($R = 0.3026$, $N = 15$) oxides from trench 7 seep soil using hydroxylamine hydrochloride and ammonium oxalate. Alpha-emitting radionuclides are comprised of approximately 76% ^{244}Cm , 17% ^{241}Am and 7% ^{238}Pu . Traces of $^{239,240}\text{Pu}$, ^{224}Ra and ^{228}Th were also detected.



of Mn (IV) minerals such as birnessite range up to $350 \text{ m}^2 \text{ g}^{-1}$ (ref. 8) and the surface charge of freshly precipitated birnessite is also usually high, exceeding $100 \mu\text{C cm}^{-2}$ at $\text{pH} = 8.0$ (ref. 9). The zero point of charge (ZPC or pH of zero net surface charge) for fresh birnessite and amorphous Mn (IV) oxides is often as low as $\text{pH} = 1.5\text{--}2.0$. Hence the manganese oxides have an unusually high negative surface charge and cation adsorption capacity over the pH range of most natural waters. For example, the cation-exchange capacity of MnO_2 at $\text{pH} = 8.3$ (as in seawater) is 1.5 equiv. per 100g while that of montmorillonite is only about 0.1 equiv. per 100g (ref. 10).

The identification of manganese oxides as strong radionuclide adsorbents in the ORNL disposal area has important ramifications for future radioactive waste disposal technology. Isolation alternatives for high-level waste currently include storage or disposal in deep geological formations such as salt, shale, or granite, and seabed disposal. Waste may or may not be solidified by processes such as calcination, cementation or vitrification into borosilicate or phosphate glass before burial¹¹. Because of their toxicity and long half lives, buried actinides must be effectively isolated from the environment for several hundred thousand years. We recommend that finely-ground manganese oxides be added to the high-level waste before solidification into calcine, salt cake or cement. Alternatively, it may be feasible to coprecipitate and encapsulate the waste with manganese oxide at high temperature. Deep-sea manganese nodules, which may in the future be mined for their valuable trace metal contents¹², could serve as a plentiful and inexpensive supply of actinide adsorbent for use in future radioactive waste disposal operations.

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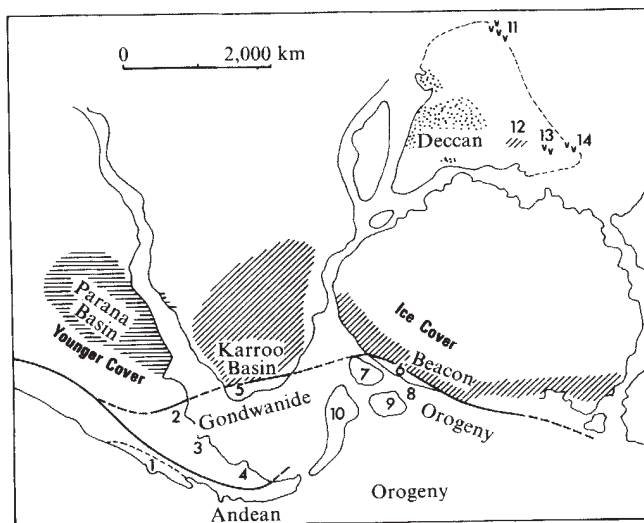
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Flood basalts, subduction and the break-up of Gondwanaland

THE hypothesis of Du Toit¹ on a continuous zone of orogeny and sedimentation (the Samfrau geosyncline) along the Pacific side of Gondwanaland in Palaeozoic and early Mesozoic times has been supported by subsequent geological work. As originally conceived, the orogenic zone of the Gondwanide orogeny consisted only of a Triassic fold-belt seen, for example, in the Cape Fold Belt of South Africa and the Sierra de la

Ventana in Argentina (see Fig. 1). Fragments of the belt are also known from the Antarctic continent in the Pensacola Mountains, Ellsworth Mountains, and the Antarctic Peninsula². Radiometric dating has established that the Gondwanides also include metamorphic and plutonic rocks, exposed in South America in the Patagonian and Deseado massifs, and in the Cordillera Frontal of Argentina (see ref. 3). These have their Antarctic counterparts in the Antarctic peninsula, Eights Coast and Thurston Island, Maria Byrd Land, and at scattered exposures between the Ellsworth and Thiel Mountains² and in eastern Australia. Several of the occurrences listed are now caught-up within the late Mesozoic-early Cainozoic Andean orogenic zone. In terms of sedimentation, the Samfrau geosyncline consisted of a 'foredeep' (for example, the Paraná and Karroo basins) developing from the late Silurian onwards and receiving sediment of a shallow marine or nonmarine nature until at least the end of the Trias. To the north the 'foreland proper' showed the transgression of Carboniferous and later sediments on to Precambrian basement. More recent work in West Antarctica has shown the existence of a greywacke-shale facies lying to the Pacific side of du Toit's foredeep². Craddock⁴ sees the Pacific border of Gondwanaland as an active continental margin showing successive orogenic episodes within the Phanerozoic. The orogenic process is believed to involve the interaction of the Gondwanaland continent with the Pacific plate, and an accretionary process at the continental margin has been suggested. With reference to Antarctica, Stump⁵ has raised the possibility of nearly continuous subduction from the late Precambrian to the Cretaceous. Dickinson⁶ has referred to the 'Gondwana-Tasman orogenic trend' as a zone of inferred plate consumption. With the information now available I have examined some of the relationships between the Gondwanide orogeny and its foreland in more detail, and discuss here

Fig. 1 The Gondwanide orogeny along part of the Pacific margin of Gondwanaland. The continental fit of Smith and Hallam²⁴ is used modified in the region of the Antarctica Peninsula to show the pre-drift positions of the Antarctic Peninsula, Ellsworth, and Thurston microplates according to more recent work by Barker, Dalziel *et al.*²⁵ and de Wit²⁶. Stipple, Deccan Traps (early Tertiary); horizontal shading, Paraná volcanism (early Cretaceous); diagonal shading, Karroo, Antarctic, and Rajmahal volcanism, (early Jurassic). v, the Panjal, Sylhet, and Arbor volcanics (late Palaeozoic to early Mesozoic age). 1, Cordillera Frontal; 2, Sierra de la Ventana; 3, Patagonian Massif; 4, Deseado Massif; 5, Cape Fold Belt; 6, Pensacola Mountains; 7, Ellsworth Mountains (pre-drift position); 8, area between Ellsworth and Thiel Mountains, 9, Eights Coast and Thurston Island (pre-drift); 10, Antarctic peninsula (pre-drift); 11, Pir Panjal range, Kashmir; 12, Rajmahal Traps; 13, Sylhet Traps; 14, Arbor volcanics.



whether there may be a connection between flood basalt volcanism and orogeny.

The Gondwanide foreland is the site of three of the world's largest flood basalt provinces, each of which consists of an extrusive phase together with a voluminous suite of doleritic sills penetrating the underlying platform sediments. The basalts and dolerites of southern Africa (The Karroo)⁷ and Antarctica (Ferrar Dolerites and Kirkpatrick Basalts associated with sediments of the Beacon supergroup)² are mostly of early Jurassic age and were probably erupted shortly before the Indian Ocean was established by the separation of Africa and Antarctica. The Paraná basalts are substantially younger (~125 Myr)⁸ and are precursive to the opening of the South Atlantic. A small remnant of this province is preserved in the Kaokoveld area of southwestern Africa⁹.

As du Toit remarked, one of the noteworthy features of the foreland geology of the Gondwanides is that tensional tectonic conditions developed during the Permian and Trias at the same time as compressional forces were operating in the adjacent fold belt. In southern Africa¹⁰, for example, normal faulting accompanied the subsidence of sedimentary troughs from the Permian (Ecca) through to the onset of volcanism in the earliest Jurassic. At this stage numerous dyke swarms, running in a variety of directions show the extensional nature of the tectonic regime. Even large volcanic-filled synclines such as that of Nuanetsi in Rhodesia were accompanied by extension at right angles to fold axes. In southern Africa, because of a favourable erosion level and an absence of ice cover, it is also possible to examine the relationship between extensional zones of Karroo age and basement structure. Here there proves to be a very detailed correspondence, Karroo extension being largely taken up along the Zambesi and Limpopo zones where younger Precambrian orogenic belts intersect the Archaean craton, and along the Lebombo monocline which may mark the southwards continuation of the edge of the Mozambique belt. Within the Limpopo belt itself the control of Karroo tectonics by basement structure is seen on a relatively fine scale¹⁰.

If southern Africa is accepted as a model, the foreland of the Gondwanides was at least from Permian onwards a region characterised by a general lateral expansion. Sedimentation was eventually succeeded by extraordinarily widespread basaltic volcanism (Karoo lavas and associated hypabyssal rocks probably, for example, affected an area of ~2,000,000 km²) which reached extreme thicknesses in linear zones such as the Lebombo. Tensional tectonics persisted and shortly afterwards continental fragmentation began. This stage was probably reached in the early Jurassic in southern Africa and Antarctica but the whole volcanic episode was delayed until the early Cretaceous in South America, as was the opening of the South Atlantic. Both the South Atlantic and the Indian Ocean rifts show a broad dependence on basement structure¹¹.

Elliot² has speculated on a possible connection between rifting, subduction beneath West Antarctica, and the basaltic volcanism of the foreland. The marginal basin environment^{12,13} for the western Pacific was suggested as an analogy. This needs closer inspection because the Gondwanide foreland shows several important tectonic features of the back-arc environment in that the orogeny was bounded on the continental side by a zone showing subsidence, extension, widespread basalt volcanism, and continental break-up.

There are also many differences between the two environments. Although, for example, there is a comparability of scale in a direction parallel to the postulated subduction zone, the igneous and tectonic phenomena of the Gondwanide foreland extend for 2,000–3,000 km into the continent in a direction at right angles to this. Existing models of back-arc spreading, however, normally consider phenomena extending for only a few hundred kilometres in this direction. Additionally, when continental separation did take place within Gondwanaland, it occurred along basement-controlled lines lying at high angles to the orogenic zone, in contrast to the western Pacific where separation characteristically takes place relatively close to and

parallel with the trenches. However, the spatial relationships of Fig. 1, and the time relations discussed, suggest the strong possibility of a genetic connection between the flood basalts and the orogenic process. Hence, despite the differences between the Gondwana foreland and the western Pacific back-arc environment a hypothesis that the flood basalts are related to subduction seems to be worthy of serious consideration.

This suggestion is highly speculative but it does prompt the query whether other flood basalt provinces might have a connection with subduction. Elsewhere, within Gondwanaland, there is some intriguing evidence; for example, in the Pir Panjal range at the southern edge of the Himalayas in Kashmir (see Fig. 1) the extensive Panjal Trap sequence indicates a considerable episode of basaltic and andesitic volcanism running from the Carboniferous through to the Trias and reaching its peak in the Permian. Wadia¹⁴ comments that "a region of quiet marine sedimentation was converted into a great theatre of volcanicity, whereby an enormous superficial extent of the country was converted into a volcanic region such as Java and Sumatra in the Malay archipelago of the present day". Northern India also saw the eruption of the supposedly andesitic Sylhet Traps and Arbor volcanics at about the same time^{14,15}. Thus, on the extreme northern border of Gondwanaland there may have been a mirroring of the events previously discussed in the south. This could imply that a subduction zone developed dipping southwards under India, and that as the continent subsequently migrated northwards part of the Tethys ocean floor was subducted beneath it. Whether this might be related to the eruption of the Rajmahal Traps (see Fig. 1) during the Jurassic or to the eruption of the Deccan Traps in the early Tertiary is a matter for future consideration.

To speculate on the Siberian Traps¹⁶ or the North Atlantic Province is beyond the scope of this paper. In the latter case, a connection with any nearby orogenic zone of appropriate age is not obvious. However, if attention is restricted to the Mesozoic and younger provinces the most important remaining Northern Hemisphere occurrence, the Columbia River Plateau, shows close spatial relationship with an orogenic zone, and indeed has already been interpreted in terms of subduction processes¹⁷. Thus, the flood basalt provinces outside Gondwanaland may provide evidence in favour of the hypothesis, but the data at the moment seem to be equivocal.

The close relationship in space and time between the varied tectonic and igneous phenomena of the Gondwanide orogeny and its foreland imply a genetic relationship. No discussion would be complete without considering the possible mechanisms, which must introduce a more speculative element, but should not obscure the original factual observations.

Most mechanisms^{12,13,18–20} suggested for back-arc spreading depend on the existence of a subducted lithospheric slab below the zone of spreading. Most tacitly assume that the subducted slab disappears at the bottom of the Benioff zone at a depth of about 700 km, and that the slab does not extend under the adjacent plate for more than about the same distance. Disappear, here, means that the subducted materials become indistinguishable from the surrounding mantle. However, the cessation^{21,22} of earthquake activity in subducted lithosphere at a depth of 700 km does not necessarily mean that the subducted material ceases to exist as a definable unit beyond this point. The material may remain distinct from the surrounding mantle because of its motion, its temperature, or its composition. In examining existing models of back-arc spreading it must be remembered that they are mainly specifically designed to explain phenomena within a few hundred kilometres of the trench. The induced convection cell model of Toksöz and Bird, for example, derives its motive power from the movement of the subducted slab in the Benioff Zone itself and, hence, does not produce effects at a distance of more than about 700 km from the trench. They conclude that subduction beneath continental crust could not induce secondary spreading because the heating effect of the rising limb of the secondary convection cell is insufficient to thin the original continental

lithosphere significantly. They do, however, envisage the possibility of a slow and diffuse extension of the continental lithosphere represented by faulted provinces such as the Basin and Range, a province which had earlier been interpreted²³ as being specifically related to subduction. I conclude that if the Toksöz and Bird model has any relevance to complete continental separation as seen in the Gondwanide foreland then it requires a larger scale than presently envisaged, both in terms of geometry and time.

The Oxburgh and Parmentier mechanism is based on a completely different principle, though there is no reason to believe that it could not act in conjunction with an induced secondary convective cell. The model postulates that mid-ocean ridge volcanism creates a depleted (harzburgitic) layer in the upper part of the oceanic lithosphere. After subduction and heating the depleted layer becomes buoyant relative to the surrounding rocks and separates into a series of diapirs which rise into the overlying upper mantle and lithosphere. The attraction of this model is that the depleted layer is compositionally distinct, and, unless some very thorough process of mantle homogenisation exists, is unlikely to lose its identity at the bottom of the Benioff Zone. Several thousands of kilometres of Pacific oceanic crust have been subducted since the beginning of the Mesozoic⁶, and the record of orogeny in Antarctica indicates that the process may also have operated during the Palaeozoic. Thus, by the time fragmentation of Gondwanaland began, early in the Mesozoic, very large volumes of oceanic lithosphere may already have been subducted around its Pacific margins. It is possible that the continual emplacement of potentially buoyant depleted lithosphere underneath the continental margin was responsible for the foreland phenomena commented on, and even ultimately for the initiation of continental break-up. It is difficult to escape the conclusion that long-continued subduction is likely to displace some of the subducted material further and further underneath the adjacent plate, and hence phenomena essentially related to the subduction process might appear far into a continental interior. If volcanism is so induced its petrogenetic relationships are likely to be complex and difficult to predict. Rising harzburgite diapirs presumably supply the energy for volcanism but not the substance. The volcanic rocks seen at the surface might originate from envelopes of less-depleted mantle dragged up by rising diapirs, or by decompression during periods of lithospheric fracturing. The extension and eventual separation of the continental crust is presumably a response to the intrusion of numerous diapirs. This type of mechanism provides a plausible explanation of the more or less undirected tensional tectonics of the Karroo period in southern Africa, the lines of ultimate complete lithospheric failure and continental separation being controlled by existing basement trends. Apart from matters of scale already discussed, this latter point may be another essential difference between the break-up of Gondwanaland and the present geology of the western Pacific arcs and marginal basins. If subduction takes place along a continental margin which already possesses a pronounced structural grain parallel to the margin, as is the case round much of the circum-Pacific belt, back-arc spreading may produce marginal basins behind narrow continental slivers such as New Zealand. Furthermore, there may be a subsequent strong tendency for such basins to close. Alternatively, if strong structural grain in the foreland runs at a high angle to the subduction zone, as it does, for example, in the Mozambique belt of south eastern Africa, separation of the continental crust may produce an elongated oceanic basin which further subduction tends to widen rather than close up.

The final question is the extent to which these speculations can be tested. Regional geological studies will ultimately show whether the relationships seen in southern Gondwanaland are repeated elsewhere. Of more immediate concern is the assessment of the very large amount of geochemical data now available, much of it unpublished, for the flood basalt provinces

of Gondwanaland. It will be very interesting to find out whether geochemical zonation fits into any sort of pattern consistent with the subduction hypothesis.

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Infracambrian glaciogenic sediments from Sierra Leone

THE Rokel River Group¹⁻³ is a sequence of generally marine sediments and interbedded volcanic rocks which extends into Sierra Leone from Guinea as a NNE-SSW trending, northwards plunging synclinorium (Fig. 1). It is divided into six formations³ and rests unconformably on Archaean granitic basement. The Rokel River Group is considered to be late Precambrian to early Cambrian as it was folded and slightly metamorphosed near the present western outcrop margin, during the Rokelide orogeny^{3,4} around 550 Myr ago, the date of the Pan-African thermo-tectonic episode⁵. It is unconformably overlain by probable late Ordovician sediments of the Saionia Scarp Group^{1,6,7}. We have studied the basal formation of the Rokel River Group, the Tabé Formation³, and describe here two members, the lower one exhibiting the characteristics of a glacial deposit. These glaciogenic sediments extend the known range of the Infracambrian-Eocambrian glaciation in West Africa.

The Tabé Formation has been examined mainly in the southern half of the Rokel River Group outcrop where two members can be recognised which we call the Tibai and Dodo Members. Details of localities, stratigraphy and sedimentology of the Tabé Formation will be given elsewhere.

The Dodo Member overlies the Tibai Member in places but elsewhere it rests unconformably on the Archaean basement. It is younger than the Tibai Member but may possibly, in places, be laterally equivalent. The Dodo Member, composed of feldspathic sandstones, crossbedded in places, is considered to be of a shallow water, possibly of intertidal origin.

The Tibai Member rests unconformably on Archaean granitic basement. It is composed of three lithologies, conglomerates, feldspathic sandstones and siltstone-fine sandstone rhythmites containing dispersed granitic clasts which on

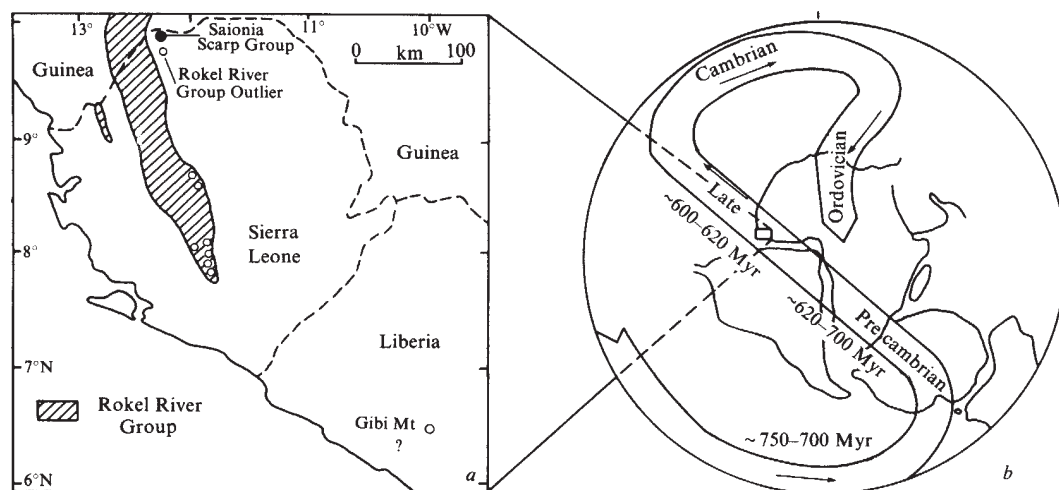


Fig. 1 *a*, Location of Infracambrian (○) and late Ordovician (●) glaciogenic sediments in Sierra Leone and possible Infracambrian glaciogenic sediments in Liberia. *b*, Pole path from 750 Myr to 400 Myr based on palaeomagnetic data (modified from McElhinney and Embleton²⁰).

a macroscopic and microscopic scale deform and depress the underlying laminations.

The conglomerates are very poorly sorted with angular to rounded predominantly granitic clasts ranging from a few mm to over 1 m in diameter. The clasts are embedded in a poorly sorted matrix of angular grains of quartz, microcline and plagioclase. Some specimens of conglomerate look like a granite which has been ground-down into angular fragments and then reconstituted and recemented by calcite.

Quartz grains were removed from the surface of specimens of conglomerate and rhythmite, using the method of Culver and Bull (unpublished) and were examined under the scanning electron microscope. In both samples, the grains show little evidence of tropical weathering and are characteristically angular. They exhibit both large (15 μ m) and small mechanically produced features in the form of conchoidal fractures and breakage blocks, but no evidence of prolonged subaqueous transport.

All the evidence suggests that the Tibai Member should be interpreted as a glaciogenic deposit; a glacial origin for the conglomerates and rhythmites was first suggested by Vallance⁸. We consider that the massive, unbedded conglomerates are tillites; scanning electron microscopy shows that quartz grain surface textures are consistent with environmental modification by a mechanical agency, perhaps glacial⁹. The petrological characters of the massive conglomerates also indicates glacial origin. Some of the thinner (30 cm) conglomerates present in the Tibai Member type section were probably not deposited directly by ice. We suggest that these conglomerates result from mass movement in the form of subaqueous mudflows¹⁰, the incorporated material, however, being generally glaciogenic.

The siltstone and fine sandstone rhythmites with isolated clasts are also considered to be glaciogenic. The randomly distributed clasts deforming underlying laminations are decisive criteria for recognition of a glacial origin^{11,12} and were supplied to the site of deposition by iceberg rafting or by a floating ice sheet. The close association of these rhythmites with the thinly bedded conglomerates further implies that the latter deposits had an original glacial origin. The massive conglomerates are present along strike, albeit discontinuously, for 225 km along the eastern margin of the Rokel River Group; thus, mudflow deposition, which tends to be localised¹³, is not consistent with the distribution of these poorly sorted conglomerates. The feldspathic sandstones, graded in places, associated with the glaciogenic orthoconglomerates and rhythmites, are considered to be turbidites; indeed, turbidite-glacial deposit associations are relatively common^{8,14}.

Glacio-marine sediments of probable late Ordovician age have been recorded from Sierra Leone^{6,7} but this is the first report of Infracambrian glaciogenic sediments in Sierra Leone.

Glacial deposits of late Precambrian to Eocambrian age have been recorded around the world¹¹ from Norway^{15,16}, Australia^{17,18}, northern France¹⁹, South-west Africa²⁰ and North-west Africa. In the latter area, tillites have been widely reported, particularly in the Taoudeni Basin²¹, the Guinea Basin²² and the Ghana (Voltaian) Basin²². Tillites also occur in eastern Senegal at the base of the Falemian orogenic belt²³, which is considered to be the northern extension of the Rokelides orogenic belt (composed of the Rokel River Group)²⁴. Thus, the presence of glaciogenic sediments in Sierra Leone extends the known range of the Infracambrian-Eocambrian glaciation (Fig. 1).

It is interesting that a poorly sorted basal conglomerate with subangular to rounded clasts has been recorded from Gibi Mt, Liberia (Fig. 1) lying unconformably on Archaean gneiss²⁵. Thorman²⁵ tentatively equated this conglomerate with that at the base of the Rokel River Group in Sierra Leone (the Tibai Member conglomerate) and we suggest here that the Liberian conglomerate may be of glacial origin.

Glacial deposits of two different ages (Infracambrian and late Ordovician) in Sierra Leone confirms the reconstruction by McElhinney and Embleton²⁰ of palaeomagnetic data concerning pole paths in the late Precambrian and early Palaeozoic. This reconstruction indicated two approaches to the West African area, one around 620 Myr ago and the other during the Ordovician (Fig. 1). The glacial deposits at the base of the Rokel River Group provide, therefore, an estimate of the time of initiation of Pan-African sedimentation in the West African area. Because late Ordovician rocks unconformably overlie the Rokel River Group^{1,6,7}, the development of the Rokel River Group sedimentary basin and the orogenic episodes associated with the Pan-African thermo-tectonic event⁵ must have taken place between 620 and 450 Myr.

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Dietary fibre in the British diet

PREVIOUS calculations¹ have shown little change in the total intake of fibre in the United Kingdom from 1880 to 1970. There has been, however, a substantial decline in the consumption of fibre from cereals, except during the Second World War. The hypothesis that relates a low intake of fibre to various diseases² refers to dietary fibre^{3,4} which includes lignin and all the polysaccharides in the diet which are not hydrolysed by the endogenous secretions of the human digestive tract⁵. However, the values for fibre used in previous calculations were for 'crude fibre'⁶, and that underestimates dietary fibre to an uncertain extent⁴. Now that values for dietary fibre in a range of foodstuffs are available^{7,8} we have repeated the earlier calculations to provide an assessment of the changes in the intakes of dietary fibre during the past century.

war years. The use of higher extraction national wheat flours from 1942 until control of the milling industry ended in 1953 led to a substantial increase in the intake of dietary fibre derived from cereals. The true value for the dietary fibre content of the 85% extraction national flours used in wartime is difficult to assess because the flour millers were under some pressure to keep the bran content as low as possible. The composition of national flour was therefore not constant as the mixing of the flour streams in the mills was altered from time to time¹¹. Consequently we have used a range of values. Southgate's⁷ figures for present-day brown flours are used for the upper value of the range of cereal fibre. A similar value (7.8 g per 100 g) can be deduced from the proportions of the parts of the grain in the national flour reported by Horder *et al.*¹¹. Analysis of present-day brown flours for crude fibre (N. Fisher, personal communication) suggests that sometimes the national flour has had a dietary fibre content nearer 6 g per 100 g. This value is used for the lower value of the range.

Table 2 shows the dietary fibre content of the average household diet calculated from the records of the National Food Survey (NFS) for selected years since 1951 (refs 9 and 10). The estimates made using the two data bases are very similar; in contrast to energy and other nutrients for which calculations from the NFS data are usually only 80% of that calculated from the total food supplies. In this respect dietary fibre may behave differently to other nutrients.

The data for 1951 indicate that the high intakes during the war years declined slowly in the immediate postwar years, and probably continued to decline until 1953 when lower extraction flours were allowed to be used. The miscellaneous sources include foods which contain dietary fibre from various sources, much of which is of cereal origin.

The changes in the composition of the dietary fibre are shown in Table 3. The increased intake of non-cellulosic polysaccharides in the war years was accompanied by smaller increases in the intakes of cellulose and lignin. Proportionately

Table 1 Dietary fibre content of the British diet

	1880	1909–1913	1938	1942	1944	1957	1970
Total dietary fibre	21.8†	23.9	22.3	32.0–37.3	34.1–39.6	23.3	22.7
From: cereals	12.2	10.9	9.2	18.7–24.0	19.1–24.6	8.7	8.1
vegetables	9.6	11.1	10.1	11.3	13.2	11.7	11.5
fruit	NA	1.7	2.7	1.9	1.8	2.6	2.6
nuts	NA	0.2	0.3	0.1	0.1	0.3	0.5
Total crude fibre*	2.1†	3.6	3.8	5.3	5.0	4.2	4.2

Figures are expressed as g per head per d and are derived from national statistics relating to total food supplies for the population as a whole^{1,9,10}. NA, not available.

* From ref. 1.

† Does not include any contributions from fruits, nuts, vegetables other than potatoes or cereals other than wheat: if the 1909 contributions from these items are included the total dietary intake is approximately 28.3 g per head per d, and the crude fibre intake about 4 g per head per d.

Table 1 shows the dietary fibre in the British diet calculated using the food supplies data described before¹. This shows that the intake of dietary and crude fibre from cereals has declined during the past century and intake from vegetables has become relatively more important. The contribution of dietary fibre from fruits has remained small and stable except for a fall in the

the increase in lignin was much greater in these components, but it is extremely difficult to measure¹². These estimates include cutin and other components which are measured with the lignin in our analytical procedures.

The greatest change of all was in the composition of the non-cellulosic polysaccharides during the Second World War.

Table 2 Dietary fibre content of the British diet

	1951†	1956	1961	1966	1971	1972	1973	1974
Total dietary fibre	25.9–28.4	22.5	21.7	21.2	21.2	20.6	20.5	20.4
From: cereals	11.1–13.6	8.4	7.5	6.8	6.4	6.1	6.1	6.1
vegetables	10.9	10.4	10.5	10.3	10.3	10.1	10.0	9.9
fruit	2.3	2.2	2.1	2.2	2.2	2.0	2.0	2.0
Miscellaneous sources*	1.5	1.6	1.7	1.9	2.3	2.4	2.4	2.4

Figures are expressed as g per head per d and are calculated from the annual national averages of household food consumption as recorded by the NFS^{9,10}.

* Includes the contribution from nuts but is mainly of cereal origin from foods containing mixtures of ingredients.

† A range is shown for this year because of the uncertainty of the actual dietary fibre content of national flours.

Table 3 Changes in the composition of the dietary fibre

	Food supplies data					National Food Survey data						
	1909–1913	1938	1942*	1944*	1951*	1956	1961	1966	1971	1972	1973	1974
Cellulose	5.7	5.4	8.1	8.7	7.3	5.7	5.6	5.3	5.2	5.1	5.1	5.1
Lignin	0.8	0.9	2.9	3.0	1.3	1.1	1.0	1.1	1.1	1.0	1.0	1.0
Non-cellulosic polysaccharides	17.4	16.1	26.3	27.9	19.7	15.7	15.1	14.8	14.8	14.5	14.4	14.3
Components of the non-cellulosic fraction												
Hexoses	11.8	10.2	12.7	13.8	10.7	9.9	9.5	9.0	8.9	8.7	8.6	8.6
Pentoses	2.5	2.6	9.0	9.3	4.9	2.4	2.3	2.4	2.5	2.4	2.4	2.4
Uronic acids	3.2	3.3	4.5	4.8	4.0	3.4	3.3	3.3	3.3	3.3	3.2	3.2

Figures are expressed as g per head per d.

* These are based on the upper values given in Tables 1 and 2. They may have been proportionately lower.

The high extraction wartime wheat flour resulted in a three- to four-fold increase in the intake of pentose, which accounts for nearly half of the increased intake of total dietary fibre between 1938 and 1942. The intake of uronic acids derived from both fruits and vegetables has not altered greatly since 1909. The increase in the war years was due to the increased consumption of vegetables. The intake of hexose also increased at this time as a consequence of increased consumption of potatoes and cereal.

The second half of Table 3 which is derived from the NFS data shows that the consumption of cellulose and lignin has now returned to 1938 levels and that the consumption of the non-cellulosic polysaccharides is now marginally lower.

Our calculations therefore show that since 1880 intake of dietary fibre has declined slowly and progressively, except during the war years when it almost doubled. Over the entire period cereal sources of dietary fibre have become progressively less important compared with vegetables. During the war years the increase in total dietary fibre was mainly due to an increased intake of non-cellulosic pentoses in the high extraction wheat flours. This would be associated with doubling of faecal weight¹³. However, in wartime fat consumption was reduced and the contribution of starch to the total carbohydrate increased, with the increased consumption of cereals. One could therefore argue that factors central to all the major hypotheses relating dietary intake to health were operating together at that time.

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The control of cell division by tension or diffusion

SEVERAL types of experiment which have been interpreted as evidence for the control of cell division by the action of diffusion boundary layers can equally well be interpreted as being due to the action of mechanical effects. The pumping experiment, and wounding experiments¹ have all been interpreted as evidence that increasing the rate of exchange of metabolites between cell and medium stimulates the cell cycle and in particular the transition G₀ to S. However, there is already much observational evidence that suggests that mechanical stress may lead to increased division^{2–4}. The pumping and wounding⁵ experiments might mechanically stress cells so that it is timely to carry out an experiment which distinguishes between the effects of mechanical tension and the effects of changed diffusion rates. We provide experimental evidence that mechanical tension may stimulate the cell cycle in vertebrate cells.

We used the technique, described previously⁶, in which cells are grown on a fabric mesh. They form sheets, 'sails', between the fibres. The sails are composed of confluent, multilayered cells which show division and which are not attached to any substrate, only other cells and the fibres. This culture can be mechanically deformed by stretching the mesh but the area of the cell sails remains constant because distortion moves one pair of sides of the mesh apart while the other two move towards each other. Thus the application of intermittent tension prevents any change in cell population density which is known to affect mitotic rate⁵.

We grew sails sheets of cells from fibroblasts from explants of 7-d-old chick embryos, cultured on nylon mesh, hole size 200 µm × 200 µm (Tobler, Ernst and Traber) in a medium of 80% Eagle's minimal medium, 10% fetal calf serum and 10% Tryptose phosphate broth. The mesh was anchored to a polystyrene ring in a culture dish. After 4 d of culture extensive sail sheets had formed. In the experimental series cultures were mechanically stressed for 1 h by deforming the mesh with the aid of a piezoelectric ceramic element (Mullard) which oscillated with a movement of 60 µm when a very low frequency sine wave (0.25–1 Hz, 120 V point-to-point) was applied.

We examined the effects of mechanical stress on mitotic rate and the proportion of cells in S. Controls provided base-line data about mitotic and S phase frequency and also checked whether the insertion of the probe caused wounding or the oscillation of the probe produced stirring which might affect mitosis and the cycle. The controls were of three types: (1) untreated cultures, no probe inserted; (2) cultures, probe inserted but not oscillated; (3) cultures with the probe stirring

the medium but not stretching the cell sheet. In the experimental series and control (3) three different frequencies of agitation were used.

After 1 h of mechanical stress or an equivalent period of stirring, control (3), or 1 h of simple culture (controls (1) and (2)) cultures were fixed, stained and the frequency of mitotic figures counted. In the second experiment the cultures were pulse-labelled during the 1 h of mechanical stress (or equivalent treatment in controls) with $2 \mu\text{Ci ml}^{-1}$ ^3H -thymidine (Radiochemical Centre, Amersham), and then chased with cold thymidine for 1 h without mechanical stress. The cultures were then prepared as whole mount autoradiographs and the percentage of labelled nuclei counted.

The results (Table 1) show that mechanical stressing of the cell leads to an increase in mitotic frequency and in the proportion of cells in S phase. The similar increases in these two different stages of the cell cycle suggest that the effect is to generally speed up the cycle rather than, say, to switch cells from G_1 to S. Subsidiary experiments in which stressing was continued for 3 h gave almost identical results, which reinforces the interpretation that stressing speeds up the cycle. Control (2) (non-moving probe) gave results which indicate that the increased mitosis or DNA synthesis in the experimental series cannot be due to wounding effects, which have been described

as stimulating growth⁹. Controls (3) (probe moving above the culture and stirring the medium only) suggest that stirring cannot be producing the stimulation seen in the experimental series. The objection could be made that stirring in the experimental series is different and more effective than in the control series. However, the mechanical effect of a probe with a tip of about $100 \mu\text{m}$ diameter moving through a distance to $60 \mu\text{m}$ at a frequency of $0.25\text{--}1 \text{ Hz}$ is very much less than that of a pump ejecting medium at a rate of $1\text{--}10 \text{ cm s}^{-1}$ in a jet 1 mm wide. In addition, different frequencies of agitation (see Table 1) in the experimental series stimulate the cell cycle either equally (compare 0.25 with 0.5 Hz) or increasingly (compare 0.5 with 1 Hz) with reduction in frequency. The latter result is the opposite of that which would be expected if stirring stimulated the cell cycle and indicates that there may be some mechanical feature of the system which determines which frequency produces greatest stimulation. If the cycle was diffusion controlled by the stirring rate it would be expected that mitotic rates and extent of thymidine incorporation would increase with the agitation rate. This is not so.

A whole series of observations and experimental results which have either been interpreted as evidence for diffusion limitation of cell division^{1,10,11} or given no particular explanation^{12,13} can be explained as being effects on the cell cycle and division resulting from mechanical stressing. For instance, the topoinhibition of division¹⁴ appears when cultures become confluent. We would expect that there would be a reduction of tension in normal cells on their becoming confluent simply because they are unable to extend themselves over each other. Since extension of cells and the generation of tension within them depends on the microfilament system¹⁴, we suggest that there must be an involvement of the microfilament system in the control of the cell cycle. The stimulation of division at the edge of a 'wound' in a confluent monolayer of cells¹ can be explained if it is assumed that the extension of cells into the newly created space allows them to retension themselves.

The anchorage dependence of division in normal cells^{11,12,15} and the fact that there is a minimum dimension of surface for this phenomenon¹⁶ can be explained by this hypothesis if it is presumed, as Maroudas¹⁶ first postulated, that the cells have to spread sufficiently to tense themselves, and that tension stimulates the cell cycle.

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Note added in proof: The recent papers by Whittenberger and Glaser¹⁷ and by Folkman and Moscona¹⁸ respectively suggest that diffusion control of the cell cycle does not occur and that cell shape may be important in growth control.

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Table 1 Effects of repetitive tensing of a cell sheet on mitotic index (Expt 1) and on ^3H -thymidine incorporation (Expt 2)

Expt 1		
Frequency (Hz)	Experiment mitotic index	Control: probe moving, not tensing cells mitotic index
0.25	4.1 ± 1.1	2.5 ± 0.4
0.5	5.1 ± 1.5	2.3 ± 0.7
1.0	3.8 ± 1.7	2.2 ± 0.4
Control, no probe inserted	2.1 ± 0.4	
Control, probe inserted, not moving	1.9 ± 0.6	
Expt 2		
Frequency (Hz)	Experiment % labelled cells	Control: probe moving, not tensing cells % labelled cells
0.25	21.4 ± 5.1	14.3 ± 1.5
0.50	22.5 ± 5.6	14.1 ± 3.2
1.00	15.3 ± 2.5	13.9 ± 1.6
Control, probe not inserted	14.7 ± 2.2	
Control, probe inserted	14.3 ± 2.2	

Counts made on counting areas of $200 \mu\text{m} \times 200 \mu\text{m}$. Mitoses were counted as mitotic figures. Results are tabulated as percentage of cells in mitosis or as percentage of cells labelled. Each measurement is expressed as the mean $\pm$ s.d. of 20 measurements in experiments and controls carried out at different frequencies, and of at least 20 measurements in the other controls. The data from the experiments and controls with moving probe were treated by factorial analysis and the means between and within experiment and control were tested for significant difference using the Q method¹⁶. All experimental means are significantly different from control means, $P < 0.005$, except for those measurements in experiment 2 carried out at 1 Hz , which are not significantly different, $P > 0.05$. The 1 Hz experimental results in the labelling experiment are significantly different from those at 0.5 Hz and 0.25 Hz , $P < 0.005$, and in the mitotic index experiment from the 0.5 Hz result, $P < 0.005$. Otherwise within experimental and within control comparisons of means are not significantly different, $P > 0.05$. The control groups in each experiment were compared amongst themselves by t -test; means are not significantly different, $P > 0.05$.

Demonstration of a cell-free factor involved in cell interactions during mating in *Tetrahymena*

CELL interactions play a major part in the development of a metazoan organism. An ideal system for studying cell interactions is one in which two and only two homogenous cell populations are involved. This condition is met in unicellular systems in which cells of two differing mating types may be induced to enter the sexual phase of their life cycle and conjugate. Conjugation in *Tetrahymena* is characterised by an association of cells of complementary mating types into pairs joined at their anterior tips. Pairing does not begin until ~1 h after mixing starved cells of complementary mating types (Fig. 1). It has recently been shown that a cell interaction called costimulation occupies this 1-h period; costimulation is required for and distinct from cell pairing^{1,2}. A block at the level of costimulation necessarily blocks pairing. Therefore, the absence of pair formation may arise from conditions which affect costimulation, or pairing, or both. We demonstrate here, for the first time, a requirement for an extracellular factor during the costimulation interaction and show that the factor behaves neither like a gamone nor like an agglutinin. Its properties suggest a role in mediating a direct interaction between cells, and involving physical proximity or contact between participating cells.

Figure 1 shows that when the starved mating types are washed and suspended in fresh buffer before mixing, pairing is delayed for >3 h. By this time control cells have already reached their plateau value. A mock wash control showed the effects of centrifugation and resuspension can be ruled out as a contributor to the loss of the ability to pair (data not shown), so it seems that something necessary for pairing is removed by the washing procedure. The necessary material is found in the cell-free starvation conditioned medium (SCM). Test cells were washed twice in fresh Tris and resuspended in combined SCM. Figure 1 shows that after mixing washed mating types reconstituted with SCM, pairing occurs with kinetics similar to those of unwashed controls. Thus, cell-derived material in SCM restores the ability to pair to washed cells.

Fig. 1 Effect of washing on *Tetrahymena* pair formation. Separate cultures of mating types B3 and B7 of *T. thermophila*¹⁰ previously known as *T. pyriformis* syngen 1 were shifted from growth medium (2% proteose peptone) to starvation medium (10 mM Tris, pH 7.4) and incubated at 28°C for 18 h at 5×10^5 cells per ml. To produce unwashed control cells (●), equal volumes of cells of the two mating types were combined at a final concentration of 1×10^5 cells per ml and 10 ml of cells were incubated at 28°C in a 125 ml Erlenmeyer flask. At 60-min intervals, 0.5-ml samples were withdrawn from each flask, fixed with an equal volume of 2% formaldehyde in 10 mM phosphate buffer at pH 7.0. Cells were scored microscopically as single or paired. Washed cells (×) were obtained by centrifuging starved cells of the two mating types twice and resuspending them twice in 10 mM Tris, pH 7.4. Reconstituted cells (○) were washed as above, but resuspended in starvation conditioned medium (SCM), collected from cells which had been subjected to separate overnight starvation. The SCM was combined from both mating types.

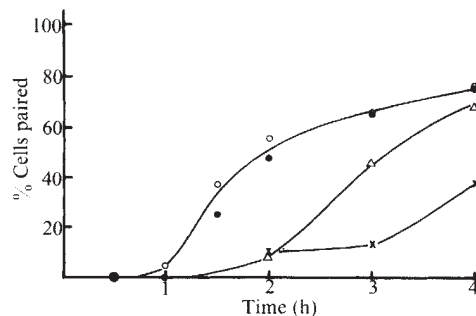
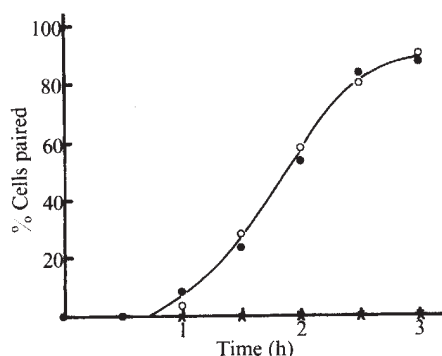


Fig. 2 Effect of SCM on costimulation. Starved cells of each mating type were adjusted to 2×10^5 per ml. One group of cells was washed and a, reconstituted with SCM at 0 time (●); b, resuspended in Tris as a wash control (×); or c, resuspended in Tris and incubated for 1 h before adding an equal volume of combined SCM (Δ). As an additional control a group of cells was mixed without washing and after 1 h of incubation an equal volume of Tris was added (○). Washed mixed cells show a 1 h lag before pairing after SCM is added, whether or not they had been incubating together for 1 h without SCM.

stituted with SCM, pairing occurs with kinetics similar to those of unwashed controls. Thus, cell-derived material in SCM restores the ability to pair to washed cells.

The experiment detailed in Fig. 2 shows clearly that the costimulation interaction, distinguished from the pairing process, does not take place without some factor from SCM. In this experiment starved cells at 2×10^5 per ml were washed, mixed and incubated for 1 h. At this point experimental samples received an equal volume of combined SCM and control samples received the same volume of Tris buffer. As additional controls (1) unwashed cells were mixed and incubated at 2×10^5 per ml for 1 h at which time an equal volume of Tris was added and (2) mixed, washed cells were reconstituted with combined SCM at zero time. Pairing kinetics in both these controls were normal, and in the sample without SCM conjugation occurred at low levels. In the experimental sample which received SCM at 1 h, a further 1 h elapsed before pairing began. Had SCM been needed only for cell pairing, then costimulation would have occurred without SCM and pairing would have begun immediately after addition of SCM. This was not the case. It seems therefore that the costimulation interaction—as evidenced by the lag period—depends on the simultaneous presence of SCM and cells of both mating types. This conclusion does not rule out the possibility of a separate role for SCM in the pairing process itself.

To establish whether a factor produced by cells of each mating type was the inducing agent in costimulation we performed two tests. First, we preincubated washed cells of each mating type in SCM derived from the complementary mating type for 1 h before mixing them. Pairing did not begin during the 1 h after the preincubated cells were mixed (Table 1, line 11)—the lag period was not eliminated. From this we infer that costimulation was not bypassed by the preincubation. Second, we incubated and washed cells of both mating types in SCM taken from one or the other mating type. Pairing kinetics were the same as those observed when combined SCM was used (data not shown; see Figs 1 and 2 for pairing kinetics). Therefore factor produced by one mating type is operationally equivalent to factor produced by the other. We conclude that medium conditioned by each mating type during starvation does not contain an inducing agent for costimulation interaction. The factor is not hormone-like, or gamone-like in its activity.

Neither is the factor an agglutinating agent. This is demonstrated by adding SCM of one mating type to washed or unwashed cells of the other mating type, and vice versa. Even after 6 h of incubation no physical association between cells of like mating type are observed in conditions in which controls had paired (Table 1).

The properties of SCM are interesting. SCM is needed for a cell interaction yet it is not the effector of the interaction as might be expected for 'hormones' in the SCM. SCM must be present simultaneously with the interacting cells, yet it does not agglutinate them. Further compounding the difficulty in understanding the mechanism of action of SCM is the fact that SCM produced by either one of the two mating types is sufficient to support pairing. The equivalency of SCMs speaks against any mating type specificity that it might have.

Activity in combined SCM is retained after dialysis suggesting that the active component is a macromolecule or macromolecular complex. Preliminary tests indicate that the activity is stable after up to 30 min of immersion in boiling water, so it is not likely that its role in pairing is due to the activity of a typical protein.

It has been previously shown that rapid agitation blocks costimulation and, independently, cell pairing itself³. The two distinct interactions therefore require positional stability. We have shown here that some component of SCM is needed for costimulation and that SCM from either mating type is sufficient. Elsewhere we show that a component of SCM is needed for cell pairing itself and that this component has specificity for the mating interactions⁴. Therefore both cell

interactions require positional stability, both require a factor—perhaps the same factor—which is supplied by either mating type.

What is the role of the factor in supporting the costimulation cell interactions? Neither a gamone role nor an agglutination role seem satisfactory. In this regard the *Tetrahymena* system is unlike yeast or *Blepharisma* in which gamones have been discovered^{5,6}. Nor is it similar to *Chlamydomonas* or *Paramecium* in which a factor or structure produced by one mating type causes massive isoagglutination of cells of the complementary mating type^{7,8}. Miyake⁹ has suggested that *Tetrahymena* cells interact through transient direct cell contacts involving surface bound effectors and receptors. This attractive hypothesis now must be modified to include a role for the hitherto unsuspected factor.

We propose that the factor serves to mediate direct interactions between cells of complementary mating types. It is possible that the factor acts passively, like an extracellular matrix, by holding cells of complementary mating types in place sufficiently long for the surfaces of the two cells to make direct contacts which may involve coupling specific effectors and receptors. This notion is consistent with Miyake's concept. Alternatively the factor could be an asymmetric molecule serving as a transiently shared effector, active only when all the components are physically linked. Both possibilities require that all three components be present simultaneously, both require positional stability that could be prevented by rapid agitation, and both allow for mating type equivalence of the factor.

It will be of interest to determine the precise means by which *Tetrahymena* cells communicate with each other and how the factor contributes to the communication. It may be hoped that some insight will be gained by knowing more about the molecular properties of the heat stable macromolecule.

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Table 1 SCM contains no isoagglutinating activity

Initial treatment	Second treatment	Time at which pairing begins (min)
1. B3 mixed with B7	None	50
2. WB3 mixed with WB7	None	165
3. WB3 mixed with WB7; add SCM	None	42
4. WB3 added to SCM B7	None	—
5. WB7 added to SCM B3	None	—
6. WB3 added to SCM B7	Wash and resuspend in Tris	—
7. WB7 added to SCM B3	Wash and resuspend in Tris	—
8. WB3 incubated separately from WB7	Wash, resuspend in Tris and mix	150
9. WB3 added to SCM B7	Wash and mix with WB7	—
10. WB7 added to SCM B3	Wash and mix with WB3	—
11. WB3 added to SCM B7 WB7 added to SCM B3	Mix	60

Starved cells were subjected to the first treatment (shown in column 1) and, in the cases shown in column 2, the second treatment was performed after a 1-h incubation. Pair formation was monitored hourly for 4 h and the time at which pairing was first observed was determined from plots similar to that shown in Fig. 1. B3 and B7 indicate starved cells of mating types B3 and B7, respectively while WB3 and WB7 indicate washed, starved cells of the appropriate mating type. SCM indicates starvation conditioned medium from mating types B3 and B7 combined, SCM B3 the starvation conditioned medium from mating type B3 alone and SCM B7 stands for starvation conditioned medium from mating type B7 alone. Lines 1–3 are the unwashed control, the washed control and the washed reconstituted control, respectively. Lines 4 and 5 demonstrate that SCM does not cause isoagglutination of cells of the complementary mating type. (Since isoagglutination is not observed when the cells of each mating type are starved separately for up to 96 h in the presence of their own SCM, we concluded that SCM does not isoagglutinate cells of the mating type from which it is derived.) Lines 6–10 show that incubation of washed cells with SCM from the complementary mating type does not produce cells that agglutinate or pair after an additional wash. Moreover, incubation of washed cells with SCM of the complementary mating type before mixing the cells is not sufficient to bypass costimulation. If this had occurred the time at which pairing begins would have been significantly less in line 11 than in line 3.

Stage-specific antigens on the surface membrane of sporozoites of malaria parasites

EFFECTIVE immunisation against malaria has been achieved in hosts ranging from birds to man by repeated inoculation of irradiated sporozoites derived from the mosquito vector^{1–4}. In all host-parasite systems so far investigated both the protection against challenge^{1–3} and the concomitant antibody response^{2,5,6} are strictly stage-specific, that is, directed exclusively against the sporozoites. The question of whether ant sporozoite immunity might also develop in natural conditions in individuals in endemic malaria areas has not been investigated, except for a preliminary study which gave negative results⁷.

Monitoring for antisporezoite antibodies requires a serological test which must differentiate between antibodies directed at sporozoite versus blood stage-specific antigens and must also be highly sensitive (capable of detecting the low level of immunity which might occur in response to the small numbers of sporozoites introduced into the host by the mosquito bite). We report here the development of such a serological test in rodent and simian malaria systems, and the initial results of its application to the sera of a few individuals exposed to *Plasmodium falciparum* malaria.

Sporozoites and infected red blood cells of *P. berghei*, *P. knowlesi* and *P. falciparum*—rodent, simian and human malarias, respectively—were used in the serological reactions. Sporozoites were obtained from the salivary glands of laboratory-bred *Anopheles* mosquitoes fed on the appropriate infected hosts.

Antisporezoite sera were derived from mice and rhesus monkeys repeatedly immunised by intravenous injection of irradiated sporozoites. Antibodies against erythrocyte stages were obtained from the sera of mice which had either an acute blood induced infection, or had survived such infections and were repeatedly reinoculated with parasitised erythrocytes. The rhesus anti-erythrocyte stage antisera were obtained by immunisation of monkeys with blood stages of *P. knowlesi* emulsified in Freund's complete adjuvant.

Two serological methods, circumsporozoite precipitation (CSP) and indirect immunofluorescence (IF), were used to determine the stage and species specificity of the antibodies. The CSP reaction was performed with viable sporozoites using a previously described technique⁸. The endpoint titre recorded was the highest serum dilution which produced a thread-like precipitate on the posterior end of the sporozoites. The IF reactions were performed with viable sporozoites in suspension in order to limit antibody binding to surface antigens. This was based on the hypothesis that stage-specific antigens, essential to parasite–host cell interactions, could be expected to be localised on the parasite's surface. The reactions obtained with viable sporozoites were compared with those obtained with parasites which were air-dried and frozen before incubation with the same panel of antisera.

Table 1 shows the results of CSP and IF reactions in the *P. berghei* system using antisera of different stage and species specificities. When the reactions were performed with viable sporozoites in suspension, positive results were obtained only with antisera produced against *P. berghei* sporozoites.

The results were quite different when the IF reactions were performed with parasites whose surface membrane had been altered by air-drying and freezing prior to incubation with immune serum. Such preparations reacted not only with anti-sporozoite antibodies, but also with the sera of mice which had been infected or immunised with blood stages of *P. berghei*. This indicates that additional common antigens had been exposed during air-drying and freezing.

A considerable difference in the antibody titre in the CSP (1:20) and the IF (1:640) tests was observed. This greater sensitivity of the IF assay corroborates initial observations in the rodent system which indicated that the IF reactions were detectable earlier and remained positive for considerably longer periods than the CSP reactions (J. McNamara, unpublished).

In addition, IF and CSP reactions were performed on rodent sporozoites with heterologous anti-*P. knowlesi* antisera. Viable and preserved *P. berghei* sporozoites gave negative results or very low titres when reacted with either of the simian antisera. This is consistent with the species specificity of these reactions noted previously in rodent, avian, simian and human malarias^{9–12}.

Stage and species specific antisporezoite antibodies were also detected in the *P. knowlesi*–rhesus monkey system (Table 2). Again, intact sporozoites reacted only with antisera produced by immunisation with sporozoites of the same malarial species. Antisera produced by immunisation of the rhesus with merozoites or a mixture of sexual (gametes) and asexual erythrocyte stages reacted with the frozen but not with the intact sporozoites. The anti-erythrocyte stage antibodies interacting with frozen sporozoites, however, gave consistently lower titres than those obtained with antisporezoite antibodies. The reactions were also species specific, that is, no reaction occurred with antisera produced against another malarial species.

Recently we examined some sera of individuals living in an area highly endemic for *P. falciparum* malaria with positive IF

Table 1 Stage and species specificity of antimalaria antibodies detected with viable and frozen sporozoites and blood stages of *P. berghei*

Antiserum against:	Serum obtained	CSP titres	IF titres	
		with viable sporozoites	with viable sporozoites	with frozen sporozoites
<i>P. berghei</i> sporozoites	by immunisation (a)	1:20	1:640	1:640
<i>P. berghei</i> IRBCs*	from chronic infection (b)	Neg	Neg	1:80
<i>P. berghei</i> IRBCs	from acute infection (c)	Neg	Neg	1:160
	from normal mice	Neg	Neg	Undiluted
<i>P. knowlesi</i> sporozoites	by immunisation (d)	Neg	Neg	1:10
<i>P. knowlesi</i> IRBCs and gametes	by immunisation (e)	Neg	Neg	1:10
				1:4
				ND†

Anti-*P. berghei* antisera were obtained by bleeding groups of A/J mice. a, Mice immunised i.v. with a total of 2×10^5 irradiated sporozoites (8 doses) and protected against sporozoite challenge. b, Mice spontaneously recovered from the i.v. inoculation of 1×10^3 *P. berghei*-infected red blood cells (RBCs) and resistant to multiple reinoculation with parasitised erythrocytes. c, Mice infected with 1×10^6 parasitised RBCs and bled when parasitaemia was $\pm 20\%$. Each of the anti-*P. knowlesi* antisera was obtained from a single rhesus monkey. d, Rhesus immunised i.v. with a total of 5×10^7 irradiated sporozoites (12 doses) and fully protected against sporozoite challenge. e, Monkey immunised with 2 i.m. injections of *P. knowlesi*-infected RBCs enriched for microgametes, and emulsified in Freund's complete adjuvant (FCA). Infected RBC slides were prepared from washed *P. berghei*-parasitised mouse erythrocytes. The cells were distributed on multiple-well immunofluorescence slides and stored at -70°C before reaction with antisera. *P. berghei* sporozoites were dissected from the salivary glands of *Anopheles stephensi*, 18 days after a blood meal on an infected hamster. The sporozoites were purified on a DEAE–cellulose column¹⁶ and either used immediately as 'viable sporozoite' or distributed on multiple-well slides, air-dried and stored at -70°C , until used as 'frozen sporozoite' antigen. The CSP and IF reactions with viable sporozoites were performed in suspension by incubating the parasites with an equal volume of serum for 30 min at 37°C . For IF reactions with 'frozen sporozoites', the slides were thawed, rinsed in phosphate-buffered saline and incubated with the same serum dilutions. The sporozoite slides were fixed with acetone and incubated with a fluorescein conjugated anti-mouse γ -chain (Meloy). Reactions with simian antisera were stained with fluorescein conjugated anti-human IgG (Behring) which cross-reacted extensively with rhesus immunoglobulins. All slides were coded and examined with a 25 $\times$ Neofluar objective using a Zeiss epi-illuminated microscope equipped with standard filters for FITC fluorescence.

* Infected red blood cells.

† ND, not determined.

Table 2 Stage and species specificity of antimalaria antibodies detected with viable and frozen sporozoites and blood stages of *P. knowlesi*

Antiserum against:	Serum obtained	CSP titres with viable sporozoites	IF titres with viable sporozoites	IF titres with frozen sporozoites	IF titres with frozen IRBCs
<i>P. knowlesi</i> sporozoites	by immunisation (d)	Undiluted	1:160	1:1280	1:64
<i>P. knowlesi</i> IRBCs and gametes	by immunisation (e)	Neg	Neg	1:80	1:4096
<i>P. knowlesi</i> merozoites	by immunisation (f)	Neg	Neg	1:80	1:4096
	from normal rhesus	Neg	Neg	Neg	Neg
<i>P. berghei</i> sporozoites	by immunisation (a)	Neg	Neg	Neg	Neg
<i>P. berghei</i> IRBCs*	from acute infection (c)	Neg	Neg	Neg	ND†

Details of the antisera (a), (c), (d) and (e) are given in the Table 1 legend. f, rhesus monkey protected against homologous blood challenge following immunisation with a total of 3×10^9 *P. knowlesi* merozoites in FCA. *P. knowlesi* sporozoites were dissected from the salivary glands of infected *Anopheles balabacensis* and purified on a DEAE-cellulose column. *P. knowlesi* infected RBCs were obtained from a rhesus monkey with a 21% parasitaemia. The sporozoites and RBC antigens were prepared, incubated with antisera and reacted with the appropriate fluorescein conjugate as described in Table 1 legend.

* Infected red blood cells.

† ND, not determined.

reaction with infected RBCs. The IF reactions using viable sporozoites of *P. falciparum* were carried out at the MRC Laboratories in The Gambia, West Africa. As a positive control we used serum samples of a volunteer (G.Z.) who had been successfully immunised by the bite of *P. falciparum* infected irradiated mosquitoes³. A serum sample taken when the volunteer was resistant to sporozoite challenge and had a CSP titre of 1:40 gave an IF titre of 1:4096 with viable sporozoites. The IF reaction of this serum sample with parasitised red blood cells was not above background level. A serum sample taken shortly after this volunteer had developed patency following challenge with blood stages had an IF titre of 1:1024 with *P. falciparum* infected red blood cells while the titre with intact sporozoites remained unaltered (1:4096). Repeated absorption of this serum with *P. falciparum* infected red blood cells removed the anti-erythrocyte stage antibodies, while the IF reactions with sporozoites remained positive.

Because of limited availability of *P. falciparum* infected mosquitoes we were only able to test sera of four Gambians living in an area endemic for *P. falciparum*. All of these sera gave clearly positive IF reactions with viable sporozoites at a 1:16 serum dilution. Control sera from individuals who had never been in malarious areas, a simian anti-*P. knowlesi* sporozoite antiserum, and the serum of a volunteer successfully immunised against *P. vivax* sporozoites⁴, gave completely negative reactions.

These data lead to three conclusions. (1) Stage-specific antigens are present on the surface of sporozoites of rodent, simian and human malarias. These can be detected either by antibodies interacting with viable sporozoites in suspension, or, as we recently found, with glutaraldehyde-fixed sporozoites. (2) Sporozoites also possess antigens common to blood stages. These antigens are not exposed on viable sporozoites and can only be detected when the parasites are air-dried and frozen prior to their interaction with antibodies, presumably when the integrity of the parasite's membrane is disrupted and additional antigens exposed. Cross-reactions between air-dried blood and sporozoite stages have been previously detected by immunofluorescence in rodent and avian malarias¹²⁻¹⁴. (3) Immunisation with intact, irradiated sporozoites produces predominantly stage-specific antibodies.

A role for these surface antigens in eliciting sporozoite induced protection is suggested by the stage specificity of this immune response and the fact that resistance in mammalian hosts has been obtained only by immunisation with intact, attenuated sporozoites, but not with disrupted parasites¹⁵.

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Tumour induction with DNA of oncogenic primate herpesviruses

THE viruses *Herpesvirus saimiri* and *H. ateles* are highly oncogenic agents of New World primates. The viruses are not pathogenic in the natural host species (squirrel and spider monkeys, respectively), but induce malignant neoplastic diseases of the lymphatic system in various other primates^{1,2} and in rabbits³. Marmoset monkeys (*Saguinus* sp.) are the most susceptible animals to tumour induction, as infection with these viruses invariably results in rapidly progressing malignant lymphoma or acute lymphocytic leukaemia within a few weeks^{4,5}. *H. saimiri* and *H. ateles* are related viruses; they share common antigens⁶ and some homologous DNA sequences⁷. DNA from both viruses is infectious in permissive owl monkey kidney (OMK) cell cultures⁸. We report here that the isolated DNA from these two herpesviruses can also cause malignant tumours in animals.

We injected 18 cotton-top marmoset monkeys (*Saguinus oedipus*) with increasing amounts of viral DNA. Four animals developed malignant lymphomas within 60–97 d after transfection.

The predominant type of DNA (M genomes, M DNA, molecular weight about 100,000,000) from both viruses has an extreme intramolecular heterogeneity in base composition⁹. A region of unique DNA (L DNA, 36–38% guanine + cytosine, molecular weight 71,800,000) is inserted between two termini of repetitive DNA (H DNA, 71–75% guanine + cytosine) with variable lengths^{7,10}.

Large batches of *H. saimiri* DNA (strain 11)¹¹ and *H. ateles* DNA (strain 73)⁶ were obtained from concentrated virus that was partially purified by velocity centrifugation in sucrose density gradients⁸. About 4.5×10^{12} virus particles were lysed in 2% (w/v) sodium lauryl sarkosinate, and the lysates were heated to 60°C for 60 min. The DNA was isolated by isopycnic centrifugation in caesium chloride gradients, and the DNA-containing fractions collected from the bottom. Purity and integrity of each DNA preparation was checked using ultraviolet spectrophotometry and analytical density centrifugation.

The inoculation scheme is summarised in Table 1. *H. saimiri* DNA (0.05–10 µg) was precipitated with calcium phosphate¹² and slowly injected into 14 adult cotton-top marmosets by the intravenous, intraperitoneal or intramuscular routes in various combinations. Animals 843, 282–75 and 283–75 died 60–85 d after transfections (Table 2). Necropsy of each monkey revealed generalised enlargement of lymph nodes, hepatomegaly and pronounced splenomegaly.

Microscopic examination confirmed malignant lymphoma of the lymphoblastic type with extensive invasion and replacement of lymph nodes, spleen, bone marrow, thymus, liver and kidney. There was no increase in absolute peripheral lymphocyte count; however, in animal 283–75, 5 lymphoblasts per 100 white blood cells were present.

H. saimiri was re-isolated from each of these animals in primary kidney cell cultures or by cocultivation of peripheral lymphocytes, spleen cells or lymph node cells with OMK cells. All re-isolation trials, except one, were successful. The re-isolated viruses were identified as *H. saimiri* strain 11 by cross-neutralisation in cell culture and by comparative analysis of their DNA with restriction endonucleases *EcoRI* and *XbaI*.

Four cotton-top marmosets were infected similarly with 20 µg *H. ateles* DNA. One monkey (animal 711–76)

Table 2 Marmoset monkeys developing malignant lymphoma after infection with viral DNA

Animal no.	Animals infected with <i>H. saimiri</i> DNA			Animal infected with <i>H. ateles</i> DNA
	843	282–75	283–75	711–76
Amount of inoculum (µg)	1	10	10	20
Survival period (d)	63	60	85	97
Premortal antibody titres:				
Immunofluorescence	NT	1:40	1:40	<1:4
Neutralisation test (neutralisation index)	NT	0.5	0.5	0.0
Virus isolation				
Primary kidney culture	NT	+	+	0
Cocultivation with OMK cells of:				
Peripheral lymphocytes	NT	+	+	0
Lymph node cells	NT	+	+	0
Spleen	+	+	0	0

None of the other marmosets showed signs of a malignant disease within an observation period of at least 10 months. NT, Not tested.

succumbed after 97 d with the clinical and gross pathological features of malignant lymphoma. The lesion resembled microscopically *H. ateles* lymphoma in this species with the exception that there was extensive necrosis of the lymphocytic follicles in the spleen and lymph nodes. All attempts to isolate virus from this monkey were unsuccessful. During the incubation period of the lymphoma, isolation of virus was attempted at weekly intervals from nasopharyngeal scrapings and from peripheral leukocytes by cocultivation with OMK cells for 4 months. Tissue fragments of spleen, thymus, lymph nodes and kidneys collected at necropsy, were explanted on OMK cells. A well established epithelial kidney cell culture was obtained. No cytopathic changes occurred in any of these cultures during an observation period of 60 d.

Pre-inoculation sera, sera collected in the incubation period, and sera obtained at autopsy were screened at a dilution of 1:4 or 1:5 for the presence of antibodies against viral structural components using published procedures^{13,14}. None of the specimens contained detectable antibodies. We searched for *H. ateles* specific DNA sequences in tumour organs of animal

Table 1 Inoculation of *H. saimiri* and *H. ateles* DNA into cotton-top marmosets

Animal number	DNA inoculation	Amount of DNA inoculated (µg)	Inoculation route (% total inoculum)			Development of neoplastic disease
			Intravenous	Intraperitoneal	Intramuscular	
874	<i>H. saimiri</i> DNA	0.05	50	—	50	0
911	<i>H. saimiri</i> DNA	0.5	80	20	—	0
843	<i>H. saimiri</i> DNA	1	80	20	—	+
769	<i>H. saimiri</i> DNA	2	70	—	30	0
1030	<i>H. saimiri</i> DNA	4	—	50	50	0
901	<i>H. saimiri</i> DNA	4.1	35	25	40	0
497–7	<i>H. saimiri</i> DNA	4.2	50	25	25	0
497–8	<i>H. saimiri</i> DNA	4.2	—	50	50	0
879	<i>H. saimiri</i> DNA	4.5	5	50	45	0
829	<i>H. saimiri</i> DNA	5.4	80	20	—	0
858	<i>H. saimiri</i> DNA	6.5	—	53	47	0
556	<i>H. saimiri</i> DNA	8.1	24	38	38	0
282–75	<i>H. saimiri</i> DNA	10	50	25	25	+
283–75	<i>H. saimiri</i> DNA	10	50	25	25	+
711–76	<i>H. ateles</i> DNA	20	40	40	20	+
712–76	<i>H. ateles</i> DNA	20	40	40	20	0
731–76	<i>H. ateles</i> DNA	20	40	40	20	0
732–76	<i>H. ateles</i> DNA	20	40	40	20	0

DNA was diluted in HEPES or phosphate-buffered saline^{10,12}, and CaCl₂ was added to a final concentration of 125 mmol⁻¹. After 10 min, fine calcium phosphate precipitates formed. The suspensions were injected slowly through 20G 1-inch syringe needles.

711-16 by DNA-DNA hybridisations as described previously¹⁵. DNA was extracted from spleen and liver autopsy material and hybridised with radioactive L DNA from *H. ateles*. As shown in Fig. 1, the kinetics of re-association showed that at least 0.013% of the liver DNA and 0.015% of the spleen DNA hybridised with L DNA of *H. ateles*. As normal somatic DNA of cotton-top marmosets does not contain endogenous cellular sequences homologous to the L DNA of oncogenic primate herpesviruses (unpublished observations), the amount of viral DNA found in these biopsies was equivalent to an average of at least seven viral genome copies per diploid liver cell and eight copies per spleen cell. It is not known at this time in what state the viral DNA was present in the cells. The bimodal re-association curves suggest that possibly part of the viral L DNA sequences were not present in this tumour DNA.

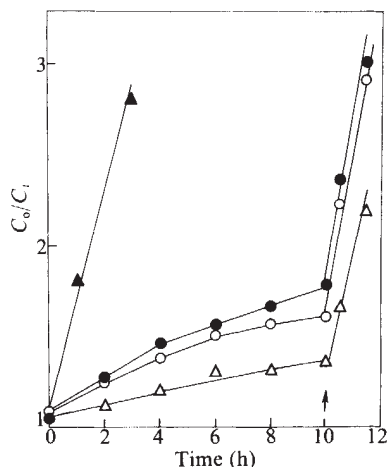


Fig. 1 Hybridisation of ³H-labelled L DNA from *H. ateles* (▲, 95 ng ml⁻¹, 340,000 d.p.m. μg⁻¹) with 2 mg ml⁻¹ unlabelled DNA from liver (○) and spleen (●) autopsy materials, compared with self-re-association (△) and re-association in the presence of 2 μg ml⁻¹ purified unlabelled L DNA from *H. ateles* (▲). In all reactions, total DNA concentration was adjusted to 2 mg ml⁻¹ with calf thymus DNA. DNA from spleen and liver accelerated the initial re-association significantly. After 4–6 h of hybridisation, velocities decreased, possibly indicating that the majority of viral genome copies in the tumour cells did not contain the total genetic information of the virus. The bimodal re-association kinetics was not an artefact due to decomposition; addition of unlabelled purified viral L DNA (2 μg ml⁻¹) after 10 h resulted in a significant increase in re-association velocity, indicating that hybridisable DNA sequences were still present at that time. C₀/C_t = d.p.m. in total DNA/d.p.m. in single stranded DNA fraction.

Spontaneous neoplastic diseases in marmoset monkeys are rare, and malignant lymphomas have never been observed in the species *S. oedipus*, except after infection with oncogenic herpesviruses. The neoplastic diseases described here seem to be causally related to the inoculation with viral DNA, as infectious virus was re-isolated from tumour-bearing animals or high amounts of viral DNA were found in autopsy materials. However, there was a striking difference in the incubation period between those tumours and lymphomas after inoculation with infectious virus particles. The neoplastic diseases described here appeared after an unusually extended incubation period of 60–97 d. In cotton-top marmosets infected with *H. saimiri* or *H. ateles*, the majority succumbed to malignant lymphoma within 30 d. The long incubation period after transfection with *H. saimiri* and *H. ateles* DNA shows that either transformed cells or biologically active DNA can persist in the organism over prolonged periods.

In the animal with lymphoma after inoculation of *H. ateles* DNA, we were not able to re-isolate virus or to demonstrate an immune response to structural viral antigens. This is in contrast to lymphomas caused by infectious virus particles, where the virus is readily recoverable and antibodies are produced. This could indicate that the lymphoid tumour cells did not contain the complete *H. ateles* genome. Cells from herpesvirus-associated tumours (Epstein-Barr Virus, *H. saimiri*, *H. ateles*, Marek's disease virus) usually contain intact viral genomes. Possibly, the neoplastic disease after transfection with *H. ateles* DNA followed a different pathogenesis. This would suggest that the total genetic information of an oncogenic herpesvirus does not necessarily need to be present to initiate malignant growth, and this could be related to the observation that *H. saimiri*-transformed cell lines sometimes contain only episomes of defective viral genomes¹⁶. However, it should be noted that the apparent lack of detectable virus in one animal does not prove that this is a general feature of *H. ateles* DNA transfections in cotton-top marmosets. These monkeys are an endangered species, and because of logistic problems it will not be possible to extend the series of DNA inoculations to reach statistical significance.

The ability of herpesvirus DNA to induce tumours indicates that DNA molecules of large size can be incorporated into the cells of the living organisms in higher mammalian species. It also emphasises the potential risks of applying DNA-containing herpesvirus vaccines to people.

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Mouse chromosome 5 codes for ecotropic murine leukaemia virus cell-surface receptor

INFECTION of cells with murine leukaemia virus (MuLV) strains involves an initial interaction between the major viral glycoprotein (gp71) and specific cell surface receptors¹. Virus adsorption and penetration occur only in cells having specific receptors². Other cellular factors such as virogene integration sites may also be required for productive infections. There are three major classes of MuLV: ecotropic viruses which replicate preferentially in murine cells; xenotropic viruses which do not replicate in mouse cells but can replicate in cells from other species; and amphotropic viruses which replicate in mouse cells as well as in cells from other species. None of the three classes of virus infect hamster cells^{3,4}. Previous studies with hamster × mouse somatic cell hybrid clones segregating mouse chromosomes showed that replication of ecotropic and amphotropic viruses required a cell function(s) coded by genes assigned to mouse chromosomes 5 and 8, respectively³. However, the function(s) of these genes was not determined. The third class of MuLV, the xenotropic viruses, could not be tested in this system since both parent cells were resistant⁵. In the present study, the replication patterns of ecotropic and amphotropic strains were correlated with the ability of hamster × mouse hybrid clones to bind gp71 of Rauscher leukaemia virus (RLV), an ecotropic strain of MuLV. By comparing the mouse chromosome segregation patterns of hybrid clones which retained the gp71 binding ability with those clones which had lost it, we have been able to assign the gene(s) coding for RLV cell surface receptor to mouse chromosome 5.

Eighteen hybrid clones losing mouse chromosomes were generated by fusing Chinese hamster lung E-36 cells⁶ with freshly isolated spleen or bone marrow cells from 6-week-old

Table 2 Correlation between mouse chromosomes and ¹²⁵I-gp71 binding to hamster × mouse hybrid clones

Mouse chromosome	No. of clones tested	Chromosome retention/ ¹²⁵ I-gp71 binding			
		No. of primary clones with:			
		+/+	-/-	+/-	-/+
1	11	4	2	2	3
2	11	5	3	1	2
3	11	4	3	1	3
4	10	2	2	2	4
5	9	5	4	0	0
6	11	5	3	1	2
7	11	5	1	3	2
8	11	3	3	1	4
9	10	5	3	0	2
10	10	3	2	2	3
11	11	0	4	0	7
12	11	7	1	3	0
13	11	4	0	4	3
14	10	3	4	0	3
15	11	7	0	4	0
16	11	7	3	1	0
17	11	6	1	3	1
18	11	4	4	0	3
19	11	4	3	1	3
X	11	7	0	4	0
Y	11	3	3	1	4

Parallel cultures of hybrid clones were examined for mouse chromosome content and tested for ¹²⁵I-gp71 binding at the same cell passages. Staining with fluorescent benzimidazole dye (Hoechst 33258)¹⁰ was used to estimate the number of mouse chromosomes present, and a modified trypsin-Giemsa method¹¹ was used for the identification of all Chinese hamster (E-36) and mouse chromosomes in hybrid metaphases. The columns labelled +/+, -/-, +/-, and -/+ indicate the number of clones in which the compared parameters (chromosome retention/gp71 binding) were present (+) or absent (-). The number of metaphase spreads examined for each clone was 13–20. Clones were scored as positive for a given chromosome if at least 15% of the cells examined contained that chromosome; however, in the vast majority of the positive clones, 60% or more of the cells contained the given chromosome. When less than 15% of the cells of a hybrid clone contained a given chromosome, that particular chromosomal data was disregarded. Clones in which none of the metaphase spreads examined contained a given chromosome were scored as negative for that chromosome.

Table 1 Correlation between ¹²⁵I-gp71 binding and replication of murine leukaemia viruses in hamster × mouse hybrid cells

MuLV	No. of clones tested	¹²⁵ I-gp71 binding/virus replication				%Discordancy
		+/+	-/-	+/-	-/+	
Ecotropic viruses						
RLV	18	9	9	0	0	0
MLV	6	4	2	0	0	0
FLV	8	5	3	0	0	0
N- and B-tropic	7	5	2	0	0	0
Amphotropic viruses						
4070	18	7	6	3	2	28
1504	8	3	1	2	2	50

Source and methods for the preparation of ecotropic virus strains Rauscher (RLV), Moloney (MLV), Friend (FLV), N-tropic virus, and B-tropic virus, and the amphotropic viruses, 4070 and 1504, were as described previously³. The cultures were infected at multiplicities greater than one 24 h after seeding 2–3 × 10⁵ cells into 75 cm² Falcon plastic flasks. The adsorption period was 1 h at 37°C using 1 ml of concentrated virus. Virus production was determined by the FA assay 21 d after infection. Virus infections and ¹²⁵I-gp71 binding assays were done on parallel cultures. The figures in the columns labelled +/+, -/-, +/-, and -/+ indicate the number of hybrid clones in which the parameters compared (gp71 binding/virus replication) were positive (+) or negative (-). Since the *Fv-1* restriction⁹ was expressed in permissive hybrid clones retaining chromosome 4 (ref. 3), N- and B-tropic virus replication data were combined.

BALB/c, NZB, or AKR mice. Procedures for the preparation, characterisation and maintenance of hybrid cells have been previously reported⁷.

RLV replication and ¹²⁵I-gp71 binding were compared in mouse NIH/3T3, hamster E-36, and E-36 × mouse hybrid clones (Fig. 1). E-36 cells bound less than 15% of the amount of gp71 bound by NIH/3T3 cells. After RLV infection, E-36 cells did not react with fluorescein isothiocyanate-conjugated antiserum to MuLV group specific antigen and produced less than 0.1 pmol of RNA-dependent DNA polymerase (RDDP) per ml of supernatant fluid. In contrast, infected NIH/3T3 cultures were 60–100% positive by the fluorescent antibody test (FA) and the supernatant fluids contained 30 or more pmol of RDDP activity. Hybrid clones which bound 15% or less ¹²⁵I-gp71 failed to support the replication of RLV. Clones with binding values ranging from 18 to 69% supported RLV replication.

Table 1 shows that several ecotropic strains of virus replicated in all hybrid clones which bound ¹²⁵I-gp71 and failed to grow in hybrids which did not bind ¹²⁵I-gp71. No correlation was seen between amphotropic virus replication and RLV ¹²⁵I-gp71 binding. Amphotropic viruses do not cross-react with ecotropic viruses, but members within each group do interfere with each other⁸. Likewise, amphotropic viruses do not interfere with RLV gp71 binding, whereas Moloney leukaemia and N-tropic viruses do interfere¹. This implied that all ecotropic

Table 3. Correlation between mouse enzyme and ^{125}I -gp71 binding to hamster $\times$ mouse hybrid clones

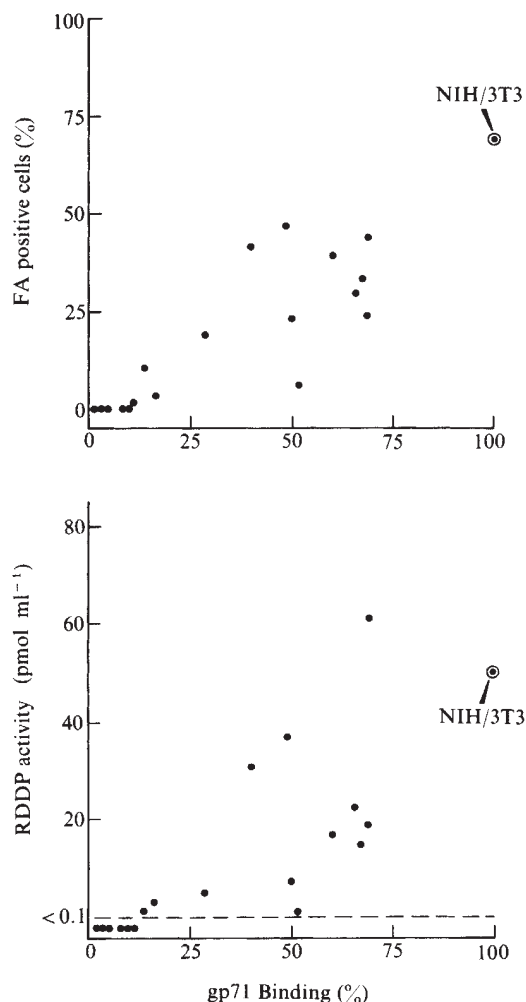
Mouse chromosome	Mouse enzyme	No. of clones tested	Enzyme retention/ ^{125}I -gp71 binding number of primary clones with:				%Discordancy
			+/+	-/-	+/-	-/+	
1	ID-1	14	4	3	3	4	50
1	DIP-1	18	9	4	2	3	28
2	AK-1	18	9	5	1	3	22
4	AK-2	17	7	4	2	4	35
4	ENO-1	18	8	4	2	4	33
4	PGD	18	8	4	2	4	33
4	PGM-2	18	7	4	2	5	39
5	PEP-S	14	6	8	0	0	0
5	PGM-1	12	8	3	1	0	8
7	GPI	18	9	3	3	3	33
7	ID-2	14	7	3	2	2	29
7	IDH-A	18	9	3	3	3	33
7	PEP-D	16	8	3	3	2	31
8	APRT	17	7	5	1	4	29
9	MOD-1	18	8	5	1	4	28
9	MPI	18	8	4	2	4	33
9	PK-3	18	8	4	2	4	33
10	HK-1	7	2	2	1	2	43
10	TRIP-1	18	9	4	2	3	28
12	ACP-1	18	10	3	3	2	28
14	ES-10	18	7	6	0	5	28
14	NP-1	15	6	5	0	4	27
18	DIP-2	15	6	5	0	4	27
X	A-GAL	15	8	2	4	1	33
X	HGPRT	16	10	0	5	1	38

The 11 hybrid clones which were tested cytogenetically and 7 additional clones were analysed for mouse enzyme expression and capacity to bind ^{125}I -gp71. Methods for enzyme separation by starch gel electrophoresis and identification of hamster and mouse enzymes were as described previously³. Correlation between cytogenic and enzyme data on these hybrid clones are published elsewhere¹².

viruses used a common surface receptor(s) which differed from the receptor(s) used by amphotropic viruses. The data presented here showing the correlation between RLV gp71 binding and ecotropic virus replication, and the lack of correlation between RLV gp71 binding and amphotropic virus replication, confirm this hypothesis.

Chromosome and enzyme analyses were performed on the hybrid clones to determine the relationship between RLV gp71 binding and individual mouse chromosomes. Table 2 shows that chromosome 5 and ^{125}I -gp71 binding segregated concordantly in all hybrid clones tested. For chromosomes 15 and 16, only one class of discordant clones was found: chromosome

Fig. 1 Correlation between ^{125}I -gp71 binding and RLV replication in hamster $\times$ mouse hybrid clones. RLV replication was determined by assays for RNA-dependent DNA polymerase (RDDP) and MuLV group-specific (gs) antigen 21 d after infection. The RDDP assay was performed using a method described previously, involving 100-fold concentrated culture fluids². Results are expressed as pmol of ^3H -dTTP incorporated into radioactive poly(dT) product per ml of unconcentrated fluid after 60 min. Detection of gs antigen was by the method of Hartley and Rowe⁸, using fluorescein isothiocyanate-labelled goat antiserum raised to Tween-ether disrupted Moloney leukaemia virus (provided by R. Wilsnack). This fluorescent antibody (FA) preparation reacts with gs antigens from all MuLV strains. The percentage of FA positive cells was determined by counting 500 cells. RLV glycoprotein (gp71) was purified, labelled with ^{125}I iodine, and assayed for binding to cells¹. Infection and binding assays were carried out on parallel cultures. Since time course studies showed that RLV ^{125}I -gp71 binding to mouse cells reached maximal levels in approximately 60 min and that NIH/3T3 cells consistently adsorbed more gp71 than BALB/c cells, the binding assay results were adjusted to the radioactivity bound by 2×10^5 test cells and expressed as a fraction of the amount bound by the same number of NIH/3T3 cells after 60 min of incubation at ambient temperature. Binding values less than 15%, FA results less than 15%, and RDDP activities less than 0.5 pmol were considered negative.



present/gp71 binding absent. These chromosomes did not segregate randomly and clones positive for gp71 binding containing chromosome 5 but lacking chromosome 15 or 16 were not found. Thus, we cannot rule out the possibility that these chromosomes may be necessary but not sufficient for gp71 binding. Chromosomes 12 and X also had the same single class of discordancy, but these chromosomes could be eliminated by enzyme analysis. In addition, a previous study using clones which had lost the mouse X chromosome following back selection in medium containing 6-thioguanine (10^{-4} M) showed that ecotropic virus replicated in the absence of X chromosome only if chromosome 5 was present. Thus, the X chromosome was neither necessary nor sufficient for ecotropic virus replication. Hybrid clones were analysed for the expression of 25 enzyme markers coded by genes assigned to 12 of 21 mouse chromosomes. Evidence from the enzyme analysis (Table 3) also excluded chromosomes 1, 2, 4, 7, 8, 9, 10, 12, 14, 18 and X. The genes coding for phosphoglucomutase-1 (PGM-1) and peptidase-S (PEP-S) have been assigned to mouse chromosome 5 (refs 13, 14). The binding of RLV gp71 segregated concordantly only with the expression of these two enzymes. In 16 of the 18 clones examined, RLV gp71 binding correlated with the presence of either PEP-S or PGM-1, or the presence of both enzymes. The remaining two clones were omitted since both clones could not be scored for either of the enzymes. One clone, which did not bind RLV gp71, lacked PEP-S but retained PGM-1, suggesting that the portion of chromosome 5 containing the gene determinants for PEP-S and the receptor for RLV gp71 was lost while the segment with the PGM-1 determinant was retained. Thus, data derived from chromosome and enzyme analyses indicate that chromosome 5 is required for RLV gp71 binding to hamster $\times$ mouse hybrid cells.

Replication of ecotropic and amphotropic murine leukaemia viruses in hamster $\times$ mouse hybrid cells requires dominantly expressed mouse genes². A genetic function(s) necessary for ecotropic virus replication was shown to be determined by mouse chromosome 5 (ref. 3). Our results indicate that chromosome 5 is necessary for RLV gp71 binding to hybrid cells. Thus, we conclude that chromosome 5 codes for the expression of ecotropic virus-specific cell-surface receptors. The failure to find clones which bind gp71 but are not productively infected suggests that chromosome 5 may also serve as the integration site. In a previous study, chromosome 8 was found to be essential for the replication of amphotropic viruses³. Whether chromosome 8 codes for the amphotropic virus receptor glycoprotein must await the development of a binding assay.

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Specific insulin binding site on T and B lymphocytes as a marker of cell activation

ATTEMPTS to identify an easily measured cell surface marker that would detect activation of T or B lymphocytes in a variety of species have been unsuccessful. Although insulin receptors have been identified for fat, liver, skeletal and cardiac muscle, fibroblasts and monocytes¹⁻⁸, quiescent rat splenic T lymphocytes do not bear such receptors. However, insulin receptors do emerge on T lymphocytes after concanavalin A treatment or allogeneic skin grafting^{9,10}. Following our work on characterising a lymphocyte insulin receptor which participates in the modulation of immune response¹¹, we have examined and report here the possibility that the emergence of a membrane-bound receptor for the peptide hormone insulin may be a universal marker for activated T and B lymphocytes.

Rat splenic lymphocyte cell suspensions were established in RPMI-1640 media buffered with 5 mM HEPES and fortified with 5% sterile, heat-inactivated fetal calf serum (designated 'enriched' media). Leukocytes were collected from a Ficoll-Hypaque gradient. The cells were fractionated on nylon wool columns yielding T-enriched, nylon wool-nonadherent cells and B-enriched, nylon wool-adherent cells. Macrophages were removed from all final cell suspensions by removing the cells that phagocytose iron particles with magnetic attraction. Insulin binding assays were carried out in suspensions containing 10^7 cells per ml in Hank's balanced salt solution enriched with 0.1% bovine serum albumin for analysis of receptor binding or the 'enriched' media for analysis of thymidine incorporation. Such purification techniques were evaluated using cell surface markers for the Fc receptor, the complement receptor, surface immunoglobulin, anti- θ determinants, and acridine orange and *p*-rosaniline staining characteristics.

The insulin receptor was measured by the use of a modification of the insulin binding assay described by Gammeltoft and Gliemann¹² which has been extensively described¹⁰. In brief, the assay involves a competitive binding reaction between radiolabelled insulin and unlabelled monocomponent porcine insulin. By such competition, both total and nonspecific isotopic binding may be measured, and specific (receptor) binding can be calculated. Specific binding is 50-60% of the total binding and is similar to that of the fibroblast. Final separation of cell-bound from free insulin was accomplished over oil with virtually no contamination of the cell fraction by free insulin and recovery of 93% of cells for analysis. Lymphocyte cultures were also evaluated for the intracellular accumulation of tritiated thymidine after a four-hour pulse of 10^6 cells in flat-bottom microtitre plates ($1 \mu\text{Ci}$ per well).

Five separate experimental protocols which result in lymphocyte activation were tested (Table 1). *In vivo* activation was accomplished by: (1) skin transplantation with histoincompatible ((Lewis $\times$ Brown Norway) $F_1 \rightarrow$ Lewis male rat) grafts; (2) graft-versus-host disease established by intraperitoneal injections of 2×10^8 Lewis splenic lymphocytes weekly for four weeks into (Lewis $\times$ Brown Norway) F_1 animals. *In vitro* activation was established in the following ways: (1) uni-

directional mixed lymphocyte cultures were established using T-enriched Lewis splenic lymphocytes as responder cells and Brown Norway mitomycin-treated thymocytes as stimulating cells. Cells were collected after three days of culture. On termination of the cultures, stimulator cells were removed by specific antibody and complement treatment and the responder cells were assessed for the presence of insulin receptors; (2) varying lymphocyte populations were cultured with the plant lectin concanavalin A (con A), phytohaemagglutinin (PHA-P), or *Escherichia coli* lipopolysaccharide (LPS).

Table 1 summarises our findings. After *in vivo* lymphocyte activation by either skin grafting or the graft-versus-host reaction, lymphocyte receptors with similar binding properties emerged. *In vitro*, T-enriched lymphocytes developed the insulin receptor after allogeneic stimulation in the unidirectional mixed lymphocyte culture or after mitogenic stimulation with con A or PHA but not LPS. B-enriched lymphocytes, but not T cells, developed the insulin receptor *in vitro* after specific T-dependent mitogen stimulation. When T lymphocytes and B lymphocytes were co-cultured with a T-cell mitogen (PHA-P), both cell populations developed the hormone receptor, probably secondary to T-B cellular interaction (data not shown). The specific binding site did not emerge after syngeneic skin grafting or co-culture with syngeneic cells. The insulin binding was saturable, specific, expressed high affinity for insulin, and was readily displaceable from the receptor. A representative binding isotherm and binding competition curve are shown in Fig. 1 for the experimental protocol using allogeneic skin engraftment.

Most polypeptide hormones convey their signals to target cells through interaction with membrane recognition units, receptors¹⁵. The criteria which established binding sites on cell membranes as specific hormone receptors include a demonstration of ligand specificity, reversibility, saturation kinetics, an affinity for hormone consistent with ligand-receptor complex formation *in vivo*, and a biological function. The T lymphocyte insulin binding site satisfies the first four conditions after activation by *in vivo* or *in vitro* stimuli. The high affinity of binding potentially allows the lymphocyte to be functionally modulated by circulating concentrations of insulin. Furthermore, binding of insulin to its putative receptor augments cytotoxic effector lymphocyte function^{10,11}. Thus, the specific binding site for insulin on the lymphocyte is a biological receptor.

Previous attempts at identification of cell surface markers that identify the active state of lymphocytes have been either unsuccessful or restrictive. The Fc receptor on the T cell¹⁴, once thought to identify the active state, has been found in several resting lymphocytes^{15,16}. Most recently, an antigen, Ala-1, has been described in mouse, occurring on lymphocytes exclusively as a result of cellular activation^{17,18}. This antigen, however, is species specific and genetically restricted with at least several alleles. In contrast, the insulin receptor on

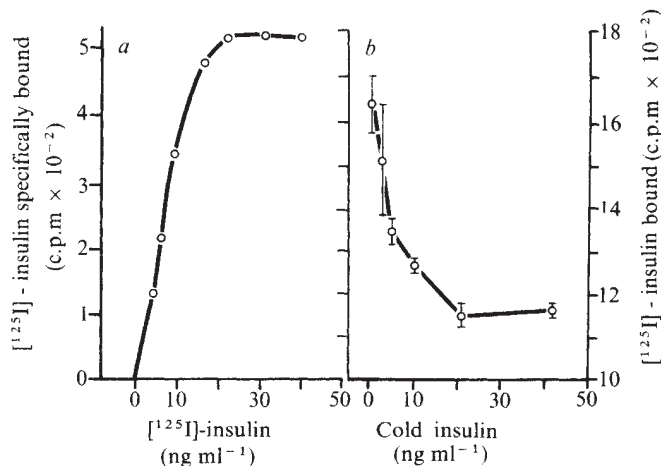


Fig. 1 A representative binding isotherm (a) and binding competition curve (b) for insulin on lymphocytes from Lewis rats 7 d after receiving (Lewis x Brown Norway) F₁ skin allografts. The half maximal equilibrium constant, calculated from the concentration of insulin at which binding is half-maximal, was 1.3 nM. The affinity of unlabelled insulin for its binding site is estimated from the concentration dependence of the binding inhibition curve²⁰ and was 0.2 nM. Both values permit binding at physiological concentrations of insulin.

lymphocytes is a marker of cellular activation which can be widely and generally applied. It is not clone specific, may be applied to activated B as well as T lymphocytes, and is not genetically or species restricted but occurs in all strains in rat and mice so far tested after manoeuvres causing cellular activation. As T-cell and B-cell mitogens selectively stimulate the appearance of T-cell or B-cell insulin receptors, these data suggest that only the subpopulation of cells that has been activated develops a receptor for insulin. Preliminary evidence from our laboratory demonstrates that the receptor also marks the active state in man¹⁹. Thus, the lymphocyte insulin receptor may be a universal marker of lymphocyte activation.

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Table 1 Appearance of specific insulin binding site on T or B lymphocytes after *in vivo* or *in vitro* stimulation

Mode of activation	Receptor occurs on isolated		Half-maximal equilibrium concentration (nM)
	T	B	
<i>In vivo</i>			
Skin graft	+	ND*	1.3
Graft-versus-host disease	+	ND*	1.5
<i>In vitro</i>			
Mixed lymphocyte culture	+	+	1.0
Mitogens			
concanavalin A	+	—	2.4
phytohaemagglutinin	+	—	2.0
lipopolysaccharide	—	+	1.5

*Not determined.

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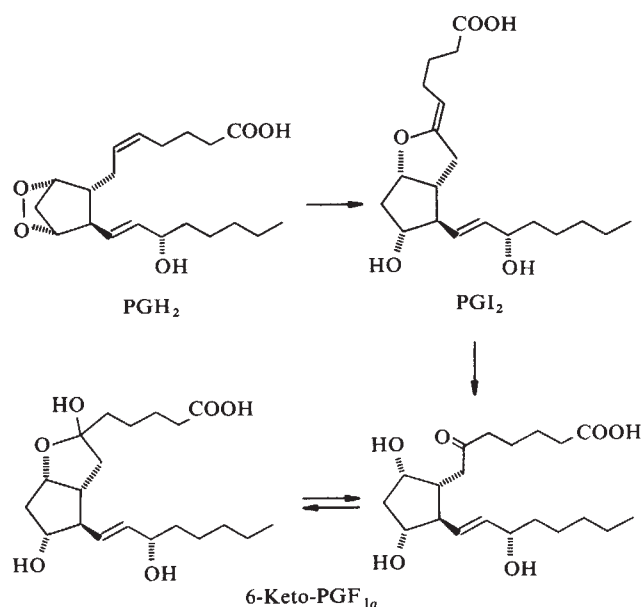
Antibodies which antagonise the effects of prostacyclin

ANTIBODIES directed against prostaglandin-albumin conjugates have been used previously as prostaglandin antagonists. Horton and Poyser¹ have demonstrated that the oestrus cycle in the guinea pig is prolonged in animals immunised against prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Ferreira *et al.*² demonstrated that rats containing antibodies to E-type prostaglandins develop a significantly reduced inflammatory oedema in response to carageenin compared with control animals. We report here that antibodies can be used to antagonise the effects of prostacyclin both *in vitro* and *in vivo*.

Prostacyclin or prostaglandin I_2 (PGI_2) is the most potent inhibitor of platelet aggregation known and produces a dramatic reduction in systemic blood pressure in the intact dog or cat³⁻⁷. This unstable prostaglandin has been shown to be synthesised by the endothelial cells of arteries and veins⁸⁻¹⁰ and its stable metabolite, 6-keto- $PGF_{1\alpha}$, has been detected in perfusates of the heart¹¹ and other tissues^{12,13}. It has been postulated that prostacyclin may be involved in maintaining the homeostasis of the cardiovascular system^{3,4} and we have now been able to test this hypothesis experimentally.

PGI_2 is produced biosynthetically by isomerisation of the prostaglandin endoperoxide, PGH_2 , but, being unstable in aqueous medium at neutral pH (ref. 14), rapidly converts to the biologically less active 6-keto- $PGF_{1\alpha}$ (Fig. 1). We synthesised PGI_2 chemically by iodination of $PGF_{2\alpha}$ methyl ester, followed by treatment with sodium ethoxide in ethanol containing 5% water¹⁵. PGI_2 was converted into 6-keto- $PGF_{1\alpha}$ by acidification to pH 5.1, and 6-keto- $PGF_{1\alpha}$ was conjugated to bovine serum albumin using 1-ethyl-3-(3-dimethyl-amino-propyl)-carbodiimide HCl (ref. 16). Two rabbits were immunised with this conjugate for a period of four months. The conjugate (1 mg) was dissolved in 2 ml Freund's complete adjuvant, an emulsion was produced by homogenisation with 2 ml 0.9% sodium chloride, and 0.2-ml samples were injected subcutaneously at multiple sites on the dorsal surface at 2-week

Fig. 1 Route of 6-keto- $PGF_{1\alpha}$ formation from PGH_2 . PGI_2 is formed by way of a synthetase present in blood vessel walls³. At neutral pH it transforms spontaneously to 6-keto- $PGF_{1\alpha}$ which can exist as a keto (right) or lactone (left) form.



intervals. The development of antibodies was followed by measuring the ability of plasma obtained from the rabbits to bind high specific activity 3H -6-keto- $PGF_{1\alpha}$ (specific activity $10.9 \text{ Ci mmol}^{-1}$). After two months of immunisation, a 1:10 dilution of the plasma bound 50% of the radioactivity. The titre rose subsequently so that after four months of immunisation a 1:1,000 dilution of plasma was sufficient for 50% binding.

Blood was withdrawn from the ear artery of normal rabbits and from each of the immunised rabbits into one-tenth volume of 3.8% trisodium citrate, and centrifuged at 180g for 15 min to prepare platelet-rich plasma (PRP). Platelet aggregation in response to ADP did not differ between normal and immunised rabbits. However, ADP-induced aggregation in PRP from immunised rabbits was only slightly inhibited by 1 mM PGI_2 , whereas the presence of 10 nM PGI_2 almost completely abolished aggregation in normal rabbit PRP. The addition of as little as 5 μ l plasma obtained from immunised rabbits to 500 μ l normal PRP obtained from rabbits, cats or man was sufficient to antagonise completely the inhibitory effects of 5 nM PGI_2 (Fig. 2).

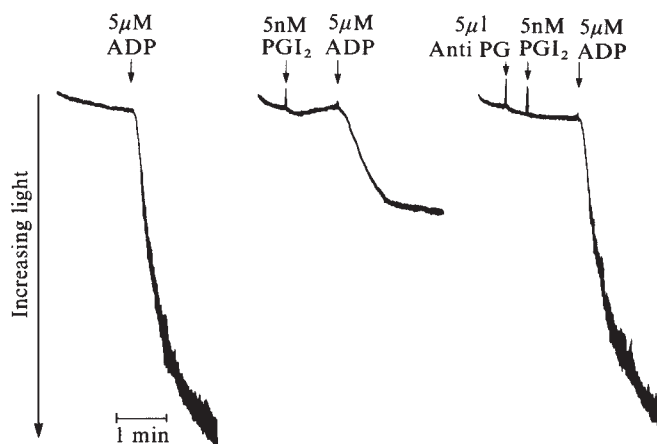


Fig. 2 Reversal of PGI_2 inhibition of ADP-induced platelet aggregation by plasma from 6-keto- $PGF_{1\alpha}$ -immunised rabbit (anti-PG). Downward deflection indicates increasing light transmission through stirred, human PRP (500 μ l) at 37°C.

Platelet-free plasma obtained from the immunised rabbits was brought to 50% saturation with ammonium sulphate to precipitate the gamma globulins. The precipitate was dissolved in distilled water and dialysed extensively against 0.9% sodium chloride. As little as 0.2 mg of this crude protein fraction completely prevented 20 nM PGI_2 from inhibiting ADP-induced aggregation *in vitro* when added before the PGI_2 . It was also effective when added after PGI_2 , although its effectiveness progressively diminished as the interval between addition of PGI_2 and protein was increased from 30 s to 5 min. At this same concentration, the protein fraction had no effect on platelet aggregation induced by arachidonic acid or a collagen suspension, but partially antagonised the inhibitory effect of PGE_1 or a stable analogue of PGI_2 , 6,9-thia- PGI_2 (ref. 17). The gamma globulin fractions prepared from rabbits containing antibodies to $PGF_{2\alpha}$ or thromboxane B_2 did not reverse the inhibitory effects of 20 nM PGI_2 even when 0.4 mg protein was tested.

Infusion of PGI_2 (1.0 nmol per kg per min) into the femoral vein of cats produced a dramatic reduction in blood pressure and a slight increase in heart rate, as reported elsewhere⁷. The simultaneous infusion of the dialysed globulin fraction from the rabbits immunised against 6-keto- $PGF_{1\alpha}$ (35 mg protein ml^{-1}) into the jugular vein caused a complete reversal of the effects of PGI_2 both on blood pressure and on heart rate (Fig. 3). This antagonism disappeared 5 min after the infusion of antibodies

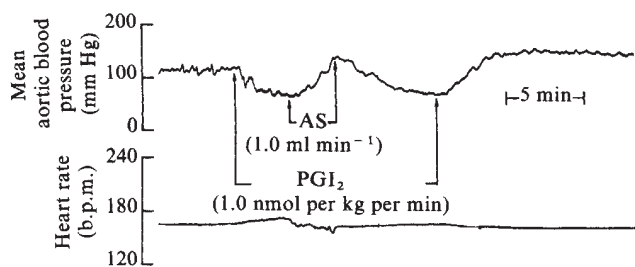


Fig. 3 Response of mean aortic blood pressure (MABP) and heart rate (HR) to infusion of PGI_2 and the dialysed globulin fraction of rabbit antiserum (AS) to 6-keto- $\text{PGF}_{1\alpha}$ in a pentobarbital-anaesthetised cat. PGI_2 produced a marked depressor and a slight tachycardia response which was totally reversed by the AS within 3 min.

was stopped. Blood pressure and heart rate returned to normal after discontinuation of the infusion of PGI_2 . Infusion of the globulin fraction obtained from rabbits containing antibodies to thromboxane B_2 (40 mg protein ml^{-1}) had little influence on the depressor effects of PGI_2 . The antibodies directed against 6-keto- $\text{PGF}_{1\alpha}$ also partially reversed the lowering of blood pressure that could be produced by infusion of arachidonic acid (500 nmol per kg per min), but did not change the depressor response during PGE_2 infusion (2.8 nmol per kg per min). In four experiments, the 6-keto- $\text{PGF}_{1\alpha}$ antibodies had no effect on the basal blood pressure in the intact cat.

The finding that antibodies directed against 6-keto- $\text{PGF}_{1\alpha}$ can antagonise the effects of PGI_2 was fortuitous. It is probable that at least some of the 6-keto- $\text{PGF}_{1\alpha}$ exists in a lactone form (Fig. 1). The structural similarity of this lactone to PGI_2 may be the reason that the antibodies exhibit cross reaction. We are now immunising rabbits with albumin conjugates of 6,9-thia- PGI_2 , in the anticipation that these antibodies will be even more active than those used in the present studies. Nevertheless, the present studies do indicate that antibodies can antagonise the effects of PGI_2 . The ability of antibodies to prevent PGI_2 from inhibiting platelet aggregation *in vivo* is predictable. The binding affinity of PGI_2 to platelets¹⁸ is at maximum $5 \times 10^7 \text{ M}^{-1}$, whereas antibodies¹⁹ are known to have affinities of 10^7 to 10^9 M^{-1} . Further studies with the antibodies should be valuable in elucidating the relationship between binding to the receptors on platelets and the associated elevation of cyclic AMP (refs 20, 21).

The reason for the short-lived effects of the antibodies in cats during PGI_2 infusion *in vivo* is not clear. One possibility is that all of the antibodies become saturated as PGI_2 is being continuously infused. However, bolus injections of PGI_2 caused identical vasodepressor effects in a cat before and 5 min after it had been infused with the antibodies. This indicates that the effectiveness of the antibodies disappears even in the absence of PGI_2 infusion. Another possible explanation is that the antibodies leave the circulation. As the antibodies were developed using a 6-keto- $\text{PGF}_{1\alpha}$ -bovine serum albumin conjugate perhaps they are being removed because they bind to the cat albumin and form antigen-antibody complexes. The production of antibodies directed against PG-polysine conjugates²² should elucidate this problem.

Our experiments indicate that PGI_2 is not a circulating vasodepressor hormone, as our antibodies, although effective during PGI_2 infusion, have no effect on basal blood pressure. The possibility remains that PGI_2 is continuously released from endothelial cells and is continuously interacting with smooth muscle cells to modulate vascular tone without ever entering the systemic circulation. Further experiments are in progress to determine whether our antibodies influence the cardiovascular effects of other hormones such as angiotensin II, bradykinin and adrenaline.

We thank Dr D. Ahern, New England Nuclear, for supplying $9\text{-}^3\text{H}\text{-PGF}_{2\alpha}$ methyl ester used in the synthesis of $9\text{-}^3\text{H}\text{-6-keto-PGF}_{1\alpha}$, and J. Komlash for assistance. This work was supported in part by grants HL21239 and HL14890 from the NIH. *Note added in proof:* Since the submission of this letter, it has been reported that antibodies directed against 5,6-dihydro-prostacyclin (PGI_1) will antagonise the effects of prostacyclin on platelets *in vitro*^{23,24}.

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Modulation of acetylcholine receptor in rat diaphragm by anti-receptor sera

ANIMALS immunised with purified acetylcholine (ACh) receptor develop a muscular weakness much like that found in the human disease, myasthenia gravis¹. This discovery led to the demonstration that myasthenia gravis is an autoimmune disease involving the production of antibodies against skeletal muscle ACh receptor²⁻⁵. Endplates from myasthenic patients have reduced miniature endplate potentials⁶, are less sensitive to ACh^{7,8} and have decreased amounts of ACh receptor⁹⁻¹¹, much of which is bound by antibody^{10,12}. In addition, there are substantial alterations in the ultrastructure of the synapse which may contribute to a loss in synaptic function¹¹. We have previously demonstrated that sera from myasthenic patients reduce the ACh sensitivity of human skeletal muscle growing in tissue culture¹³. Our experiments demonstrated that this reduction in ACh sensitivity is due to two processes; an inactivation of ACh receptor function by antibody binding¹⁴ and a decrease in ACh receptor density resulting from an antibody-induced increase in the rate of ACh receptor degradation¹⁴⁻¹⁶. The stimulation of ACh receptor degradation by antibody is energy and temperature dependent¹⁴ and is similar to the phenomenon of antigenic modulation found in other systems¹⁷⁻¹⁹. Our previous experiments apply only to the ACh receptor found on muscle grown in tissue culture, a recep-

tor which is presumably analogous to extrajunctional ACh receptor found in denervated muscle *in vivo*. Here we demonstrate that anti-receptor antiserum increases the rate of ACh receptor degradation in innervated as well as in denervated rat diaphragms maintained in organ culture.

Female Lewis rats (1–3 months old) were injected in the thoracic cavity with ^{125}I - α -bungarotoxin (αBT)²⁰. αBT is a small protein (molecular weight 8,000) which binds specifically and with high affinity to skeletal muscle ACh receptor²¹. The diaphragms were removed 14–16 h after injection, and isolated hemidiaphragms were placed into organ culture. The muscles were maintained in Dulbecco modified Eagle's medium plus various sera (see Figs. 1 and 2) supplemented with 50 mg l^{-1} insulin plus 35 mg l^{-1} *p*-aminobenzoic acid, and rocked at 37°C according to Berg and Hall²². Denervated hemidiaphragms were obtained by sectioning the left phrenic nerve under ether anaesthesia 8–12 d before the ^{125}I - αBT injection. The rate of ACh receptor degradation was determined by measuring the rate of release of ^{125}I into the culture medium. This has been shown to be an accurate measure of the turnover of extrajunctional ACh receptor^{23,24}.

Our previous experiments showed that sera taken from rats immunised with purified ACh receptor and applied to muscle *in vitro* reduced the extrajunctional sensitivity of denervated diaphragm muscle to ACh²⁵. Figure 1 shows that sera taken

from rats immunised with ACh receptor increase ACh receptor degradation in denervated rat diaphragms taken from nonimmunised animals. The data in Fig. 1 also show that sera from animals immunised against ACh receptor from either rat, fetal calf or *Torpedo californica* are all capable of modulating the ACh receptor on rat diaphragm. This demonstrates that rat ACh receptor has one or more determinants that cross-react with *T. californica* and calf and are capable of participating in antigenic modulation.

That the release of ^{125}I is due to ACh receptor degradation rather than the dissociation of ^{125}I - αBT from muscle is shown by the finding that more than 85% of the released material is of low molecular weight, as it elutes in the included volume of a BioGel P2 column (J.M., S.H. and J.L., in preparation). Furthermore, the release of ^{125}I is blocked by inhibitors of energy metabolism (5mM dinitrophenol plus 2mM NaF, see Fig. 2).

The ACh receptor at the neuromuscular junction differs in several ways from the receptor found in extrajunctional regions of denervated muscle. Endplate receptor is metabolically more stable^{26,27} and has different physical, biophysical and pharmacological properties^{28–30} which could affect its response to

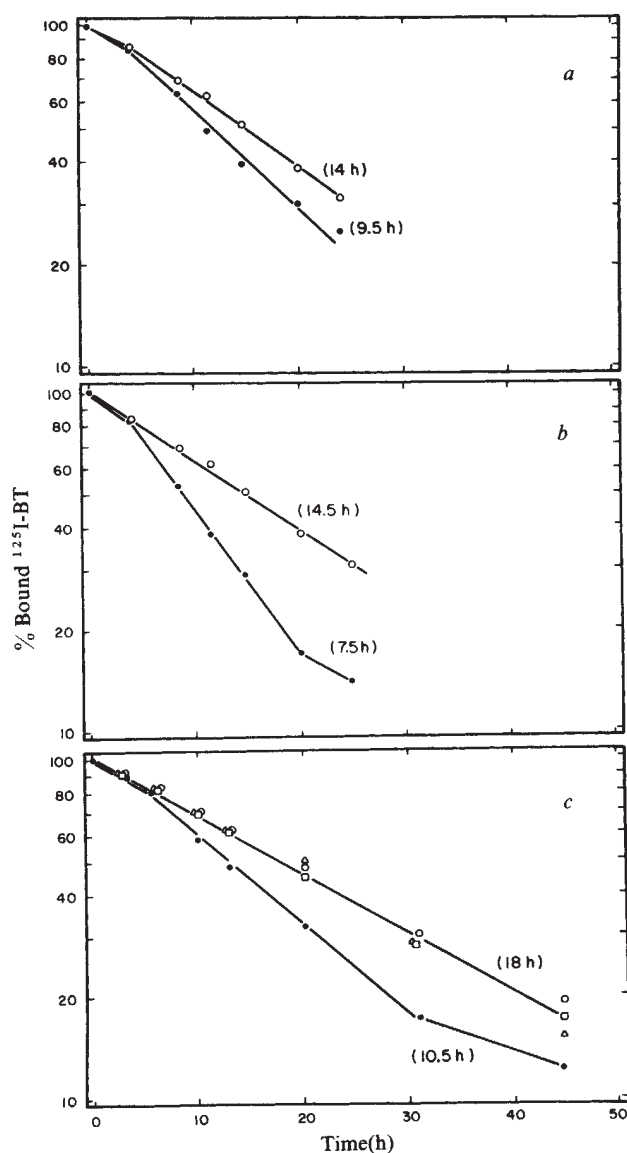


Fig. 1 ACh-receptor degradation in denervated normal rat diaphragms treated with anti-receptor sera. The left hemidiaphragm of 4–5-week-old female Lewis rats was denervated by nerve section under mild ether anaesthesia. After 8–12 d, rats were injected intrathoracically with $0.2\text{--}0.8 \times 10^{-9}\text{ mol}$ of ^{125}I - αBT ($1\text{--}2 \times 10^5\text{ Ci mol}^{-1}$) (ref. 5). Diaphragms were removed 14–16 h later and the individual hemidiaphragms cultured according to Berg and Hall²⁰. Specificity of labelling was demonstrated by Triton X-100 extraction of several diaphragms at the beginning of organ culture and precipitation of the ^{125}I - αBT -labelled receptors with anti-receptor serum^{5,35}. >90% of the radioactivity present in the diaphragm met these criteria of specificity. Furthermore, it was determined by labelling the Triton extract with saturating amounts of ^{125}I - αBT followed by radioimmune assay, that the *in vivo* labelling procedure labelled 10–20% of the total toxin-binding sites. The rate of ACh-receptor degradation was determined by measuring the rate of release of ^{125}I into the medium. In all cases the rate of release from extrasynaptic regions was derived by subtracting the rate of release from a normal (innervated) hemidiaphragm from that of a paired denervated hemidiaphragm. As the amount of ^{125}I - αBT initially bound to the muscle was 5–10-fold greater in the case of the denervated hemidiaphragm, and the uncorrected rates of release from the respective sides were approximately 10-fold different, this correction made a small but significant difference in the calculated values for $t_{1/2}$, the values of which are given in parentheses. The rate constant for degradation, K_d may be calculated from the relationship $K_d = 0.693/t_{1/2}$. To determine the effect of anti-receptor antisera on the rate of receptor degradation, the toxin-labelled hemidiaphragms were treated in culture with several sera. The denervated sides were cultured in the presence of 5% rat antiserum to purified rat ACh-receptor (a), rat antiserum to purified foetal calf ACh receptor (b), or rat antiserum to purified *T. californica* ACh receptor (c). In each case the paired innervated hemidiaphragm as well as a control pair of innervated and denervated hemidiaphragms were cultured in the presence of 5% nonimmune rat serum. All sera were heat inactivated at 56°C for 15 min to inactivate complement. Titres of antisera to immunise antigen and rat ACh-receptor were determined by radioimmune assay^{5,35}. These were: a, anti-rat ACh-receptor serum, $7.6 \times 10^{-8}\text{ M}$; b, anti-fetal calf ACh-receptor serum, $1.2 \times 10^{-5}\text{ M}$ for foetal calf receptor and $7.1 \times 10^{-7}\text{ M}$ for rat receptor; c, anti-*T. californica*, $2.1 \times 10^{-5}\text{ M}$ anti-Torpedo receptor and $3.2 \times 10^{-7}\text{ M}$ anti-rat receptor. $\circ$, $\triangle$ and $\square$, diaphragms treated with nonimmune rat serum; $\bullet$, diaphragms treated with anti-receptor sera. The effect of specific antisera on receptor degradation in these as well as previous experiments¹⁴ occurred after a lag of several hours. It is assumed that the delay is the time required for diffusion and binding of antibody to the ACh receptor. Processes involved in the degradation of ACh receptor (that is, patching, internalisation and degradation) may also contribute to the observed lag in ^{125}I release. Thus, the halftimes of decay are based on the measured release of ^{125}I that occurs after this lag ($\geq 8\text{ h}$).

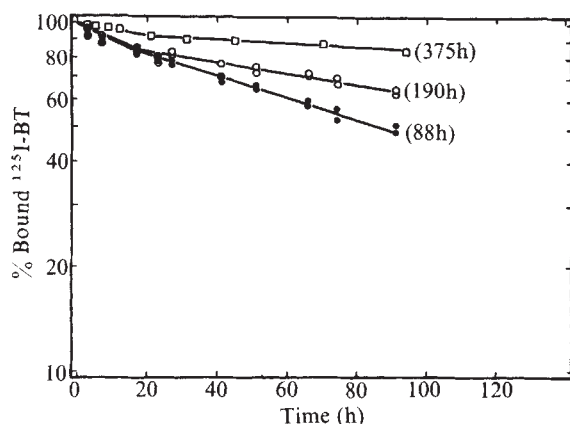


Fig. 2 ACh-receptor degradation in normal innervated rat diaphragms. Diaphragms of 5–6-week-old female Lewis rats were labelled and established in organ culture as described in Fig. 1. Paired hemidiaphragms were treated with 5% nonimmune or rat anti-*T. californica* ACh-receptor serum. One hemidiaphragm was incubated in the presence of 5 mM dinitrophenol (DNP) and 2 mM NaF. The rate of release of ^{125}I -radioactivity was determined. The values in parentheses are uncorrected $t_{1/2}$'s of total ^{125}I released. The halftimes become slightly longer if a correction is made for the small amount of undegraded ^{125}I - αBT (10–15%) that is released during the experiment. $\square$, DNP, F⁻ treated; $\circ$, nonimmune serum; $\bullet$, anti-receptor serum. The values in parentheses are calculated from the slower component of the decay curves (17–91 h). The component with the faster rate of decay, observed at early times (0–17 h) constitutes 10% or less of the total ^{125}I - αBT . This could be due to the rapid release of a small amount of unbound toxin and/or the degradation of a small population of extrajunctional receptor (which may persist in adult muscle, see ref. 31).

antibody. Figure 2 demonstrates for the first time that ACh receptor on innervated muscle is turned over. The long half life of approximately 7–8 d is consistent with earlier estimates^{26,27}. Figure 2 shows that anti-receptor sera increase the rate of ACh receptor degradation on innervated muscle approximately twofold. Control experiments demonstrated that the release of ^{125}I is energy dependent (Fig. 2) and that 85–90% of the released ^{125}I is the result of ^{125}I - αBT degradation (that is, runs in the included volume of a BioGel P2 column, J.M., S.H. and J.L., in preparation). Additional experiments demonstrated that more than 90% of the ^{125}I - αBT is initially bound specifically to ACh receptor, as it is precipitated by anti-receptor antibody (see legend to Fig. 1). ^{125}I - αBT is not degraded when incubated with muscle in the presence of excess unlabelled αBT , consistent with the idea that degradation of ^{125}I - αBT is dependent on the binding of αBT to the ACh receptor. Experiments to be published separately (J.M., S.H. and J.L., in preparation) show that the factor in serum responsible for the increased rate of ACh receptor degradation is anti-receptor antibody.

These experiments demonstrate that antisera against the ACh receptor increases ACh receptor degradation on normal rat diaphragms when applied to the muscle *in vitro*. The fact that innervated diaphragms respond to antisera by increasing their ACh receptor degradation suggests that endplate ACh receptors are subject to antigenic modulation. However, there remains the possibility that the released ^{125}I comes from extrajunctional ACh receptors present in innervated rat diaphragm, as it has been reported that a small number of extrajunctional ACh receptors exist in muscle from 5-week-old chicks³¹. We consider this possibility unlikely, as the degradation rate we find on innervated muscle is very slow ($t_{1/2} > 7$ d), consistent with endplate ACh receptor. Furthermore, antisera stimulates the degradation of more than 50% of the ACh receptors found in innervated diaphragm (Fig. 2).

Our observed increase in ACh receptor degradation induced by anti-ACh receptor antibody is complement independent, for

we have used serum depleted of complement by heat inactivation (Fig. 1 legend and ref. 14). However, muscle from patients with myasthenia gravis as well as rats immunised with purified ACh receptor have the C3 component of complement bound to their membranes^{2,32}. Furthermore, animals injected with purified ACh receptor or animals injected with syngeneic anti-receptor antibody develop an acute transient phase of muscle weakness³³, which occurs within a week of injection and involves a phagocytic attack on the postsynaptic membrane which is complement dependent³³ and is clearly different from the phenomenon of antigenic modulation described here.

Therefore, our experiments demonstrate that antiserum against the ACh receptor increases the rate of ACh receptor degradation in rat diaphragm. This finding extends the phenomenon of antigenic modulation from tissue cultured cells to muscle tissue in organ culture. In addition, preliminary results show that the ACh receptors in innervated as well as denervated diaphragms taken from rats with experimental myasthenia gravis (immunised against purified ACh receptor) are also degraded at an accelerated rate (J.M., S.H. and J.L., in preparation). It is clear that antigenic modulation results in a reduction in ACh receptor density^{14,16} and decreased muscle ACh sensitivity^{13,14,25,35} which could contribute to the symptoms of myasthenia gravis.

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Antibody to acetylcholine receptor increases degradation of junctional and extrajunctional receptors in adult muscle

MYASTHENIA gravis is a disease of impaired neuromuscular transmission (for review see ref. 1), which probably results from a reduced number of acetylcholine (ACh) receptors in the muscle membrane². Myasthenia is thought to be an autoimmune disease¹, in part because most myasthenia patients have circulating antibody to the receptor^{3,4}. Specific antibodies can increase the turnover and reduce the amount of surface proteins⁵, including ACh receptors⁶⁻⁸, on cells in tissue culture. Because such an effect could contribute to the decrease of ACh receptors found in myasthenia, we have investigated the effect of anti-receptor antibodies on the turnover of ACh receptors in innervated and denervated muscles of adult rats. Two forms of ACh receptor are found in mammalian muscle. Junctional receptors are densely packed and highly localised at motor endplates⁹⁻¹¹, while extrajunctional receptors are found in developing and denervated adult muscles at low density over the entire muscle surface¹⁰⁻¹². The two forms of receptors are almost identical molecules but can be distinguished both *in situ* and after purification (for discussion, see ref. 13). Differences in isoelectric point¹⁴ and immunological properties (ref. 15 and unpublished results of C.B.W. and Z.W.H.) suggest that they have slight structural differences. One important difference between these forms of receptors is their stability in the membrane. Extrajunctional receptors undergo continual turnover with a half-life of about 14-18 h in both developing and denervated adult muscles¹⁶⁻¹⁸. In contrast junctional receptors are much more stable and have not previously been shown to turn over, although their half-life *in vivo* has been estimated to exceed 6 d (refs 16 and 18). We report here that junctional receptors can turn over and that antireceptor antibodies increase the degradation of both junctional and extrajunctional receptors in adult muscle.

Rats were injected in the thorax with ¹²⁵I- α -bungarotoxin and normal or denervated hemidiaphragms were cultured with ribs attached¹⁶. Turnover of ACh receptors was measured by following the appearance of radioactivity in the medium. BioGel P-2 chromatography¹⁶ was used to separate ¹²⁵I-tyrosine, the product of toxin degradation, from intact toxin. The rate of degradation of toxin bound to extrajunctional receptors has been shown to be nearly identical to that of receptor alone^{17,19}. For most experiments, either control or immune rabbit serum was added to the medium. Antisera were raised against highly purified receptor²⁰ from denervated rat muscles by methods to be described elsewhere. The antisera had identical titres against both purified junctional and extrajunctional receptors and thus could be used to compare the effects of antibody on receptor stability.

Extrajunctional receptor degradation in denervated muscles had a single exponential time course¹⁶ (Fig. 1) which was unchanged by the presence of normal serum (Table 1). Addition of anti-ACh receptor sera to the cultures increased the rate of loss of radioactivity after a lag of several hours (Fig. 1). The half-time obtained for turnover in control muscles was 13.8 ± 0.7 h (mean $\pm$ s.e.m., $n = 10$); after treatment of muscles

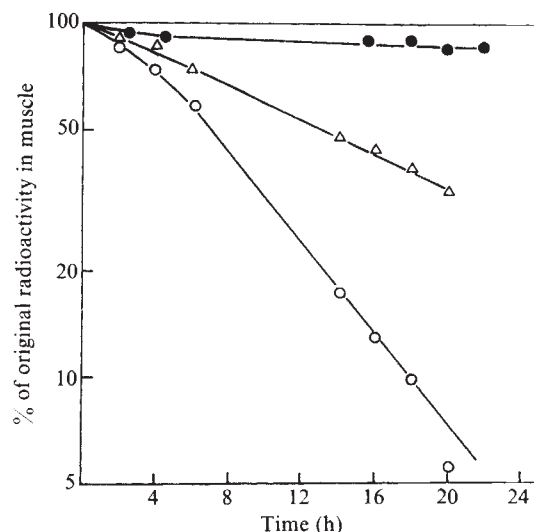


Fig. 1 Loss of radioactive toxin bound to extrajunctional receptors. Male rats (80-90 g) whose left hemidiaphragms had been denervated 5 d earlier, were injected in the thorax with 5 μ g per 100 g body weight of ¹²⁵I- α -bungarotoxin (100 c.p.m. per fmol, prepared as before²³). Three hours after the injection, the denervated and innervated hemidiaphragms were separately placed in organ culture in 5 ml of Trowell's T8 medium. After the medium had been changed three times at 30-min intervals, the appropriate serum and, in some cases, cyanide was added to the medium. The rate of turnover of extrajunctional receptors in denervated hemidiaphragms, corrected for loss from the corresponding innervated hemidiaphragm, was obtained as described¹⁶. Curves were fit by linear regression. Δ , 5% normal serum ($t_{1/2} = 13.6$ h); $\circ$, 5% antiserum ($t_{1/2} = 4.8$ h); $\bullet$, 5% antiserum + 20 mM NaCN ($t_{1/2} = 143$ h). The half-times of degradation given here and in the text for extrajunctional receptors were not corrected for dissociation, since the correction would be negligible.

with antisera, the half-time was changed to 5.0 ± 0.4 h ($n = 10$). Antiserum heated to 55° for 30 min to inactivate complement caused a similar increase of turnover. Control experiments showed that the antibody-stimulated loss of radioactivity was blocked by cyanide (Fig. 1) indicating that, like normal turnover^{16,17}, it occurred by a process requiring cellular energy. At least 90% of the radioactivity lost from the muscles in the presence of antiserum cochromatographed with iodotyrosine on columns of BioGel P-2. Thus antiserum accelerates the energy-dependent degradation of extrajunctional receptors in denervated adult muscles.

To investigate the turnover of junctional receptors, we labelled normally innervated muscles with higher specific activity ¹²⁵I- α -bungarotoxin to compensate for the smaller amounts of toxin bound, and cultured them at later times after toxin injection to reduce background radioactivity. In these conditions, radioactivity was lost from the muscles at an exponential rate (Fig. 2). The half time of loss seen either in the absence of serum or with preimmune serum was about 145 h (Table 1). Addition of cyanide to the medium decreased the rate of loss, increasing the half-time to 320 h. At least 80% of the material lost from cyanide-treated muscles eluted from BioGel P-2 as intact toxin, and therefore presumably represents dissociation of toxin-receptor complex. In untreated muscles, however, more than 60% of the radioactivity in the medium eluted as iodotyrosine. We conclude that this radioactivity comes from toxin bound to junctional receptors because both dissection of the diaphragm into junctional and extrajunctional regions¹⁶ and autoradiography of single fibres¹¹ indicate that at least 90% of the toxin was initially bound at the endplate. Thus, an energy-dependent breakdown of toxin bound to receptor can be seen in normal muscles.

The rate of loss of radioactive toxin from normal muscles was increased in medium containing antiserum against the ACh

receptor (Fig. 2). In these experiments the mean half-time of loss was about 90 h for both untreated and heated antisera. The half-time of loss was 220 h for cyanide-treated muscles. The difference between cyanide-poisoned muscles treated with normal or immune sera suggests that one effect of the antiserum is to increase the dissociation rate of toxin-receptor complex; however, the difference of half-times may not be significant ($0.05 < P < 0.10$). In any case, this effect is not large enough to account for the increased loss of toxin from unpoisoned muscles that are treated with antibody. Analysis by BioGel P-2 chromatography showed that at most 20% of the increase in loss of radioactivity caused by treatment of the muscles with antibody could be accounted for by increased loss of intact toxin. Most (80%) of the increased radioactivity eluted in the iodotyrosine peak. Thus antireceptor antibodies increase degradation of the junctional ACh receptor.

To obtain half-times that represent degradation of ACh receptors, the rates of toxin loss must be corrected for loss by dissociation. The half-time measured after cyanide treatment can be used for this correction if it is assumed that the loss of toxin seen in the presence of cyanide represents dissociation of the toxin-receptor complex and that dissociation is unaffected by inhibition of energy production. The half-time of degradation obtained in this way is 262 h (~11 d) for normal muscles (Table 1). Because of possible heterogeneity in the tissue (see legend to Table 1) and because it has not been demonstrated that turnover of junctional receptors is unaffected by toxin, this must be taken as a provisional value for the half-life of junctional receptors in the postsynaptic membrane. This is significantly longer than the lower limit for turnover time estimated from experiments *in vivo* in which it was not possible to separate dissociation and degradation^{16,18}.

The half-time for turnover of junctional receptors in the presence of antibody was found to be 158 h by this method. Thus treatment with antibody increases the apparent rate of degradation by about 65%. Even in the presence of antibody,

Fig. 2 Loss of radioactive toxin bound to junctional receptors. Male rats (80–90 g) were injected in the thorax with 10 µg per 100 g of body weight of ¹²⁵I-α-bungarotoxin (500 c.p.m. per fmol). After 18 h, hemidiaphragms were placed in culture as described in Fig. 1. Aliquots were removed from the medium at the times indicated and replaced with fresh medium. A complete change of medium was made at 24 h. At the conclusion of the experiment, total radioactivity remaining in the tissue was determined and the rate of loss from each muscle was computed. Curves were fit by linear regression. Note the compression of the abscissa compared with Fig. 1. Δ, 5% normal serum ($t_{1/2} = 137$ h); ▲, 5% normal serum + 20 mM NaCN ($t_{1/2} = 377$ h); ○, 5% antiserum ($t_{1/2} = 88$ h); ●, 5% antiserum + 20 mM NaCN ($t_{1/2} = 279$ h).

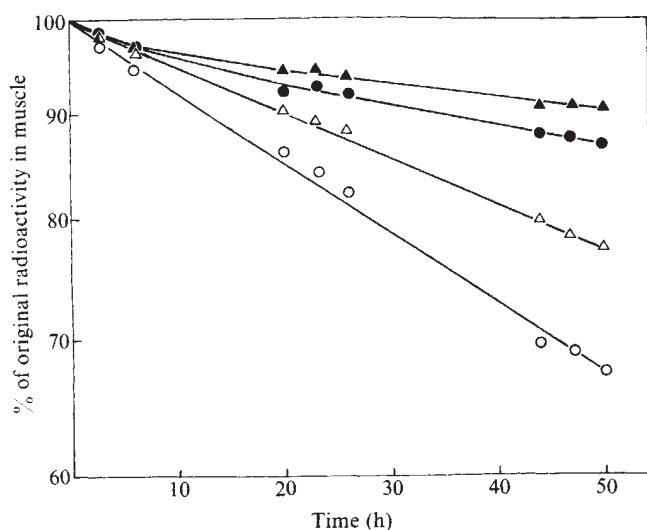


Table 1 Half-times of loss of radioactivity from junctional receptors

	$t_{1/2}$ (h)	$t_{1/2}$ with cyanide (h)	Calculated $t_{1/2}$ for degradation (h)
No serum	147 (2)		
Normal serum	142 ± 6 (3)	320 ± 24 (3)	262
Antiserum	92 ± 3 (5)*	220 ± 24 (3)	158
Heated antiserum‡	91 ± 3 (3)†		

Values represent mean ± s.e.m. (no. of muscles). Half-times were determined from best fit curves of data obtained in experiments such as those illustrated in Fig. 2. Antisera from two rabbits immunised against purified ACh receptor from denervated rat muscle²⁰ were used. Similar results were obtained with both antisera and data have been combined. The sera precipitated 0.5 nmol and 0.3 nmol per ml, respectively, of radioactive toxin-receptor complex from solution. Corrected values for the half-time of degradation were obtained by assuming that loss of radioactivity from the tissue occurs from a single pool by two processes—dissociation of intact toxin and degradation of toxin-receptor complex. The remaining radioactivity at time t can then be represented by the expression $A = A_0 \exp [-(k_1 + k_2)t]$, where A_0 is the original radioactivity in the tissue, k_1 is the rate constant for degradation and k_2 is the rate constant for dissociation. In the presence of cyanide only dissociation occurs, and the remaining radioactivity equals $A_0 \exp [-k_2t]$. Both k_2 and $(k_1 + k_2)$ can be determined experimentally and the rate constant for degradation obtained by subtraction. In the experiments on junctional receptor, about $\frac{1}{3}$ to $\frac{1}{2}$ of the radioactive toxin in the cultured tissue was bound to the diaphragm, and the remainder to the intercostal muscles and ribs. To see if loss of toxin was the same from the two sources, ribs and diaphragms were cultured separately in a few experiments, with and without antibody. The results obtained were qualitatively similar in both cases. Loss from the ribs seemed to be slightly slower than from diaphragms, but the difference was not judged large enough to warrant further investigation, and in all subsequent experiments the tissue was treated as a homogeneous source.

Significantly different from corresponding value in normal serum by Student's t test: * $P < 0.001$; † $P < 0.005$.

‡ Heated to 55°C for 30 min.

however, the turnover time of junctional receptors remains slower than that of extrajunctional receptors, and the striking difference in stability normally seen between the two receptors^{16–18} is maintained. Since the antisera used in these experiments had identical titres against junctional and extrajunctional receptors, the difference in stability presumably reflects an intrinsic difference in the receptors or in their environment.

The increase in rate of degradation that we observe would result in a steady state level of junctional receptors that is 60% of normal, if ACh receptor synthesis is unchanged by antibody. Thus anti-receptor antibody can produce a significant decrease in the number of endplate receptors. The increased receptor degradation induced by antibody could therefore be one cause of impaired neuromuscular transmission in myasthenia gravis although cell mediated responses²¹ or complement factors²² may also contribute to the pathogenesis of the disease.

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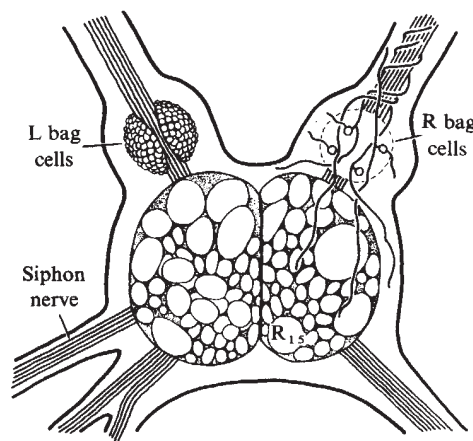


Fig. 1 Schematic diagram of the dorsal surface of the abdominal ganglion and principal nerves. The bag cells are located in two clusters, right (R) and left (L), of about 400 cells each. They send profusely branched axons into the ganglionic sheath. Some of the axons extend into regions overlying the cell bodies of other ganglion neurones, as illustrated for a few cells of the right cluster. Drawn after Kupfermann and Kandel¹⁶.

Evidence for local hormonal communication between neurones in *Aplysia*

It has been suggested that in certain systems, communication between neurones is mediated, not by conventional synaptic transmission, but by a mechanism in which substances are dispersed over relatively long distances to act on target neurones^{1–3}. The marine mollusc *Aplysia* may be useful for cellular studies of this novel mode of communication. Activation of spike activity in a group of neurones, the bag cells, produces several types of response in nearby identified cells^{4–7}. These responses have slow onsets and prolonged durations compared to those at conventional synapses. We describe here the response of one of the target neurones, cell R_{15} , which involves a distinctive change in the cell's activity lasting for as long as three hours. When the release of neurosecretory product from the bag cells is simulated by brief local application of bag-cell extract to the target cell, an apparently identical response is produced. These results, combined with previous anatomical data, support the hypothesis that the bag-cell induced responses are mediated by a local hormonal interaction, one that is intermediate between blood-borne hormonal and synaptic interactions.

Experiments were carried out on the isolated abdominal ganglion of *Aplysia californica*, dissected from animals weighing 400–600 g, and bathed in filtered blood taken from the animal at dissection. Intracellular recordings were made with 2 M potassium citrate microelectrodes using conventional recording and stimulating techniques. Detailed methods are described elsewhere⁶.

The bag cells are neuroendocrine cells^{8–10}, grouped into two bag-shaped clusters at the anterior end of the ganglion (Fig. 1)^{11,12}. They have profusely branched axonal processes that end blindly near vascular spaces in the ganglion sheath. It is presumably from these sites that a peptide hormone that induces egg laying is released into the general circulation to act on peripheral tissue^{8–10,13–15}. Some bag-cell axons end in the sheath overlying the cell bodies of other ganglion neurones, but ultrastructural studies have indicated that the axons make no synaptic contacts with the neurones^{11,12}.

The bag cells are normally silent. Following application of a brief 1–2-s train of electrical stimuli to one of the clusters, they discharge in near unison with a burst of spike activity lasting for about 20 min (ref. 16). The target cell R_{15} is a bursting pacemaker neurone; spontaneously occurring bursts of spike activity in this cell are caused by a bursting pacemaker potential (BPP) that periodically depolarises the cell^{17–19}. We found that after the start of a bag-cell discharge the BPP increased in amplitude (Fig. 2a) over a period of 5–15 min and resulted in increased burst intensity and increased number of spikes per burst (Fig. 2b). The average spike rate of the cell also increased, but there were no systematic changes in the burst frequency. Figure 2c shows averaged data plotted for target cell burst intensity for a series of experiments where stimulation produced a bag-cell burst and a series of controls where nearly identical stimulation did not produce a burst. Burst intensity developed to a plateau level 9 min after the start of bag-cell activity and was still significantly above controls at the end of the experiments after nearly 1 h. In other experiments the effect on burst intensity lasted for up to 3 h. There was no response in the controls.

The properties of the response differ in several ways from conventional synaptic responses. The onset is slow and smoothly graded, the duration is prolonged, and there are no rapid deflections in the target cell that can be interpreted as representing fast synaptic responses to the bag-cell spikes. These distinctive properties are also characteristic of the bag-cell induced responses of other identified neurones^{6,7}. The R_{15} response has an additional distinctive property; it involves a complex change in the burst-generating mechanism in which the magnitudes of both the depolarising and hyperpolarising phases of the BPP are increased. Correspondingly, we could not produce this change in R_{15} activity by either sustained depolarisation or hyperpolarisation of the cell by direct current injection.

The morphology of the bag cells and the properties of the response are consistent with a nonsynaptic mode of interaction. The response is readily obtained in ganglia that are rapidly and continuously superfused, suggesting that the interaction is not mediated by the dispersion of a hormone into the general circulation. We therefore propose that bag-cell neurosecretory product, in addition to being dispersed into the general circulation, is dispersed locally in vascular and interstitial spaces in the sheath to act on or near the cell body of the target neurone. To examine this possibility we simulated the release of neurosecretory product from the bag cells by removing the

sheath overlying the dorsal surface of the ganglion and applying bag-cell extract (BCE) locally to the cell body of R_{15} . The extract was delivered through a 50- μm polyethylene pipette at a rate of $0.02\ \mu\text{l s}^{-1}$ for 2 min while the ganglion was continuously superfused with filtered blood in a 2-ml elliptical chamber. Marker dyes showed that material pipetted in this

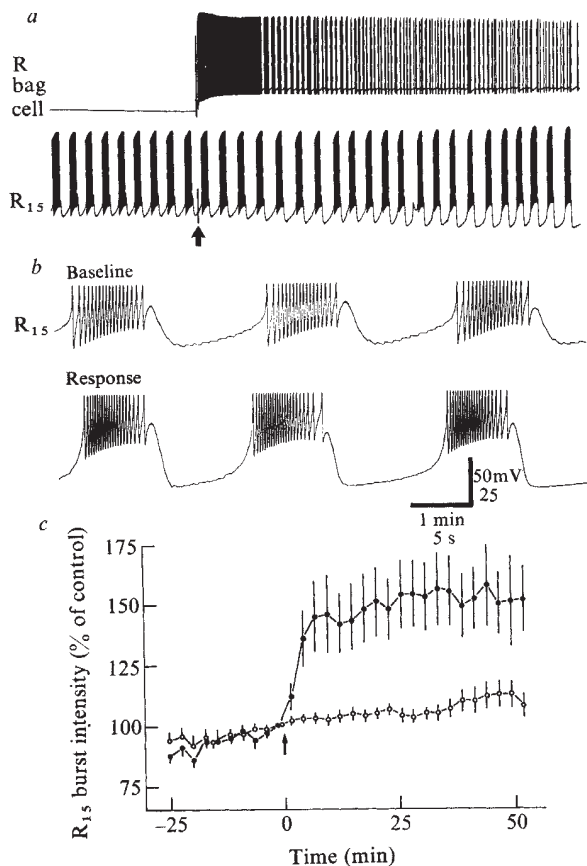


Fig. 2 Augmentation of bursting activity in cell R_{15} produced by triggered bag-cell activity. *a*, Simultaneous intracellular recordings from a right bag cell and R_{15} . Brief local stimulation ($5, 0.6\ \text{mA}, 5\text{-ms}$ pulses at $5\ \text{s}^{-1}$) of the right bag-cell cluster (at arrow) triggered a prolonged discharge of the bag cells that was monitored in one of them. Spikes within each R_{15} burst are seen fused together; the amplitude of the underlying bursting pacemaker potential (BPP) steadily increased for several minutes after onset of bag-cell activity. *b*, Examples of R_{15} activity (expanded time scale, tops of spikes not shown) during baseline control and at peak response, the latter showing an increase in the BPP and more intense bursts. *c*, Effect of stimulated bag-cell activity on R_{15} burst intensity, relative to stimulated controls. For each experiment the bag cells (either right or left cluster) were locally stimulated (arrow at 0 min) according to a fixed procedure which sometimes triggered a bag-cell burst. The procedure was to apply single 0.2-ms pulses at increasing intensities until an intensity was found at which a subthreshold response was produced in the bag cell. Using this intensity a train of five pulses at $5\ \text{s}^{-1}$ was then applied. If a bag-cell burst was triggered by the train, the data were included in the experimental group ($\bullet, n=8$). If a bag-cell burst was not triggered, the data were included in the control group ($\circ, n=7$). For each experiment, R_{15} burst intensity, measured as the peak spike rate during each burst, was taken as the second-most intense burst for each 2.67-min period and normalised to the baseline control value at 0 min. The means and standard errors (vertical bars) for the experimental and control groups are shown. There was no significant difference in stimulus intensity of experimental and control groups. Bag-cell burst duration for the experimental group was $21.5 \pm 4.6\ \text{min}$ (mean $\pm$ s.e.); temperature, 20°C .

manner formed a bolus around the target cell soma that was washed away 2–3 min after the end of the application.

Figure 3 shows an example of the response produced by application of BCE. The onset of the response was remarkably similar to that produced by bag-cell activity, although there was short-lived hyperpolarisation during the application that is attributable to a secondary component in the extract. As shown in Fig. 3c, the increase in burst intensity of the BCE-induced response, averaged for seven trials, closely paralleled that produced by bag-cell activity, and it remained well above baseline level for the full 30-min duration of the assay period. (Average spike rate also increased to a maximum of 155% of control at 12 min, as compared to 197% at 8 min for the bag-cell activity-induced response.) Blank controls failed to produce a response and siphon nerve extract, containing an amount of neural tissue equivalent to that in the BCE, produced only a slight increase in burst intensity relative to blank

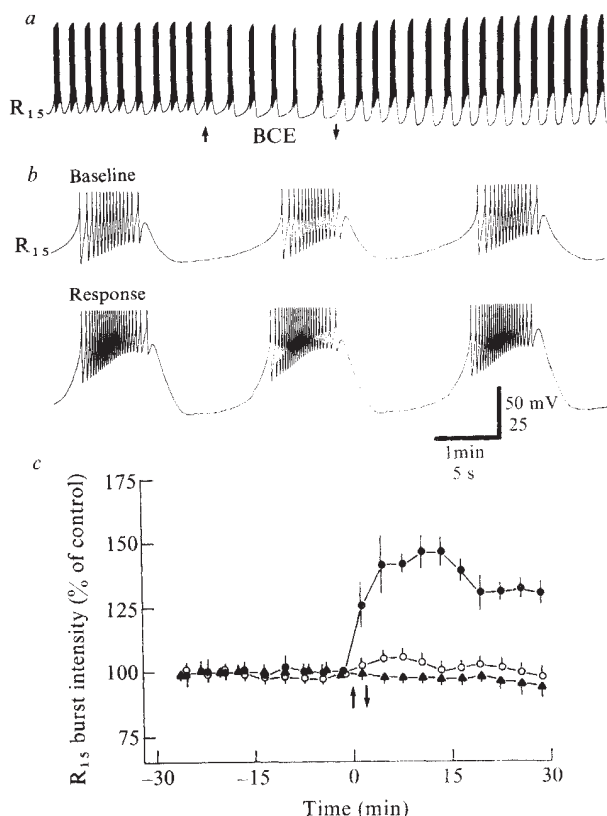


Fig. 3 Simulation of the release of bag-cell neurosecretory product by local application of bag-cell extract to cell R_{15} produces augmentation of burst activity in R_{15} . *a*, Onset of R_{15} response to 2-min application of BCE applied through a $50\ \mu\text{m}$ pipette placed near the R_{15} cell body. *b*, Examples of R_{15} activity during baseline control and peak response, the latter showing the same changes in burst activity that are produced by bag-cell stimulation. *c*, Similar 2-min BCE applications ($\bullet, n=7$) produced increases in R_{15} burst intensity that are comparable to those produced by bag-cell activity, whereas siphon nerve extract ($\circ, n=6$) and blood ($\blacktriangle, n=7$) controls were largely without effect. Data were averaged as described for Fig. 2c. For all experiments bag cells were monitored for activity, and none occurred. Bag cells from 10 animals were excised from the abdominal ganglion after rapid freezing on dry ice, and added to $150\ \mu\text{l}$ of $0.22\text{-}\mu\text{m}$ Millipore filtered *Aplysia* blood that contained $250\ \mu\text{g ml}^{-1}$ each of the peptidase inhibitors, ovomucoid and lima bean trypsin inhibitor (Worthington) and bacitracin (Sigma). The tissue was then crushed with a teflon pestle, sonicated for 15 s, briefly centrifuged, and the supernatant removed for use, or storage at -60°C . Siphon nerve controls were similarly prepared using an equivalent amount of neural tissue. Blood controls contained the peptidase inhibitors. Room temperature, $19\text{--}22^\circ\text{C}$.

controls. It is of interest that a single 2-min application of BCE nearly mimics the prolonged effects of bag-cell activity, even though the extract was washed away from the ganglion within 2–3 min after the application. Although bag-cell bursts typically last for 20 min, most of the spike activity¹⁶, and presumably most of the release of neurosecretory product, occur during the first 2–5 min of the burst, a time period comparable to the BCE application. Thus, the prolonged presence of a chemical mediator near the site of action is apparently not a requirement for the production of a prolonged response.

The simplest interpretation of the data is that bag-cell hormone acts directly on the target cell. However, because of the slow response onset we cannot completely rule out the possibility that it is produced by an indirect route through action on other nearby cells. Two other lines of evidence suggest that the action, whether direct or indirect, is on or near the soma membrane. One is that the burst-generating mechanism of R_{15} , which is markedly affected by bag-cell activity, has been shown to reside in the soma membrane¹⁹. Additionally, our preliminary studies of the mechanism of the response indicate that the response is accompanied by a large increase in the input conductance of the cell (128% increase, $n = 4$). This is consistent with the action of a hormone on a large area of membrane such as that presented by the cell body. The active factor in the BCE remains to be identified. We found that the response was not produced by bath application of acetylcholine, adrenaline, 5-hydroxytryptamine, dopamine, octopamine or histamine at 10^{-4} M concentration (see also refs 20, 21). The mediator may be a peptide, as the bag cells synthesise and release several peptides thought to be derived from a common precursor molecule^{22–24}. One of them is the egg laying hormone, a peptide of molecular weight 6,000 recently isolated from BCE using egg laying as a bioassay¹⁴. In addition, the vertebrate neurohypophyseal peptides vasopressin and oxytocin can produce a qualitatively similar response in R_{15} (refs 20, 21).

The results indicate that the bag cells produce long-lasting augmentation of bursting activity in target cell R_{15} . This augmentation might regulate homeostatic function during egg laying, as there is evidence that R_{15} is a neurosecretory neurone¹² that contains a peptide involved in the control of water balance²⁵. Our finding that the distinctive qualities of the response, including its prolonged duration, can be mimicked by brief local application of BCE to the target cell soma, strongly supports the view that the interaction is mediated by a bag-cell hormone acting locally near sites of release in the ganglionic sheath. Such an interaction involves the dispersion of the mediating substance over longer distances than transmitters at conventional synapses, but not as far as hormones that enter the general circulation. The morphology of the bag cells, their widespread effects in the ganglion, and the slow, smoothly graded characteristics of the effects suggest that all bag-cell induced responses in the abdominal ganglion may be mediated by local hormonal action. A similar mode of communication has been proposed for central aminergic neuronal systems of vertebrates. These systems, like the bag cells, have profusely branched axonal processes that do not, in general, make synaptic contacts with other neurones^{1–3}.

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Plasticity in dendrites shown by continuous GABA administration in superior cervical ganglion of adult rat

FINE structural investigations of the normal development of neurones have drawn attention to the transitory appearance of specialised postsynaptic membrane thickenings preceding the establishment of Gray type I synaptic contacts with axon terminals^{1–4}. Non-innervated free postsynaptic membrane thickenings (f-post) occur more frequently in certain developmental stages and in certain laminae of the occipital cortex than in others⁵. Temporo-spatial aspects of the cortical development^{6–8} suggested to us that the maturation of inhibitory neurones might have an important impact on the formation of f-post. We describe here our investigations on the validity of this working hypothesis by searching for structural changes at the receptive surfaces of neurones in response to extracellularly applied γ -aminobutyric acid (GABA), a substance known to act in an inhibitory way on neurones⁹. The superior cervical ganglion (SCG) was selected as a comparatively simple neuronal system which has a uniform preganglionic input and probably no neuronal feedback to the spinal cord. We found that the dendrites of the ganglion cells responded to a prolonged infusion of GABA by the formation of non-innervated postsynaptic membrane thickenings, changes in shape and the accumulation of microvesicles.

Adult rats (Sprague-Dawley) of both sexes were anaesthetised with ether and the left SCG was exposed, taking care to retain an intact blood and nerve supply. In 37 animals glass capillaries (outer diameter: 50 μ m) connected to small glass bulbs containing GABA (10 μ l of 48 mM γ -aminobutyric acid, Serva) in mock cerebrospinal fluid (CSF) were inserted into the ganglia in aseptic conditions and then fixed with tissue glue (Histoacryl blau, Braun) at the M. longus colli (for further technical details see ref. 10). The efflux of GABA into the SCG was assured by using 4-amino- n -[G-³H]butyric acid (27.2 Ci mmol⁻¹, Radiochemical Centre) and liquid scintillation counting of the solubilised SCG. The ganglia were removed three or seven days after the implantation of the glass bulbs. The tissue was fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) containing 0.5 g l⁻¹ CaCl₂, either by vascular perfusion or immersion, and processed by standard techniques for electron microscopy.

Three to seven days after the onset of the GABA infusion the structure of many dendrites had changed dramatically. Normally showing a more or less cylindrical shape and a smooth surface, the dendrites had been transformed by sending out many expansions which often penetrated the glial sheaths and made contact either with other neuronal surfaces or with the basal lamina (Figs 1b and 2). Filamentous material aggregated locally along the inner aspect of the plasma membrane

and produced structural complexes at many sites which resembled the postsynaptic membrane thickenings of Gray type I synapses (Fig. 1a). As presynaptic elements were absent at these sites, these were labelled f-post. Investigations are in progress to decide if any identifiable receptors or other characteristic proteins of functional postsynaptic thickenings are present in the f-post.

Structures similar to the f-post have been reported to occur as persistent postsynaptic membrane thickenings after axonal degeneration in the CNS^{11,12}. Although in the SCG some authors did not report them after decentralisation¹³⁻¹⁶, others provided clear evidence for their existence^{17,18}. No signs of axonal and terminal degeneration in the SCG have been seen either in the GABA-treated animals or in 10 control animals in which the implanted bulbs contained only the solvent solution (mock CSF). In the controls, only a few, isolated f-post were observed, and so their formation seems to be induced by the GABA administration.

One might interpret this result as an amplification of normal GABA activity, in response to an unphysiologically high local concentration of the amino acid^{19,20}. GABA might, therefore, also be involved in the physiological regulation of the number of synaptic contacts in the SCG.

Thus, non-innervated, so-called free postsynaptic membrane thickenings can be generated not only during perinatal synaptogenesis^{4,5}, but also by adult neurones. Their appearance and persistence do not depend on the degeneration of axon terminals or on the availability of innervating axon terminals. The formation of f-post can be induced by prolonged (>3 d) increase in GABA levels. The occurrence of f-post in normal development as well as in GABA-treated SCG suggests that synaptogenesis consists in general of at least two separable

Fig. 1a Detail from a superior cervical ganglion of rat 3 d after continuous GABA administration. The dendrite is irregular in shape, it contains many microvesicles (mv) and has a non-innervated membrane thickening (large arrow). rer, rough endoplasmic reticulum. ($\times 64,000$). **b**, A free membrane thickening (arrow) facing the glial sheath at higher magnification. D, dendrite. ($\times 110,000$).

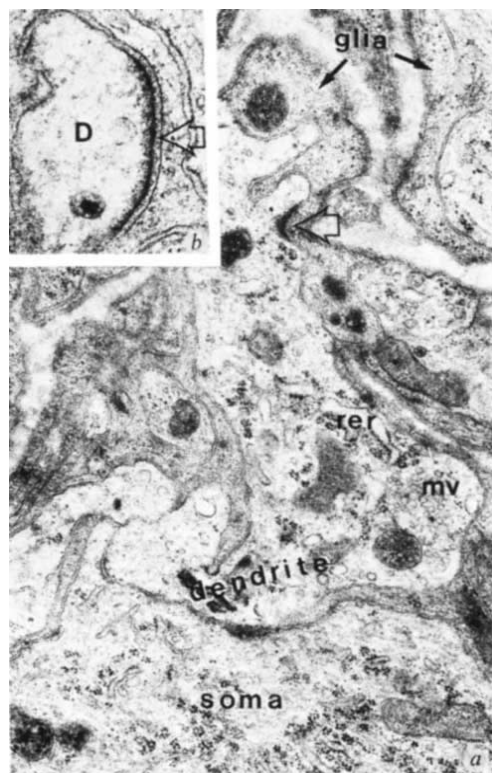


Fig. 2 Several non-innervated membrane thickenings (arrows) and a desmosome connecting two dendrites (D) are seen. Star indicates a preparational artefact. ($\times 64,000$).

processes: the formation of axon terminals or any other kind of presynaptic element, and the formation of postsynaptic sites. The actual activity of synaptogenesis would then be regulated by the probability of temporo-spatial coincidence of these two different events. Long-lasting persistence of non-innervated postsynaptic sites in the cerebellar cortex^{21,22} and in the experimentally changed adult SCG does not obviously induce axon sprouting. If GABA, however, had induced the described changes in the SCG by inhibiting the ganglion cells, then axons innervating inhibitory local circuit neurones could, by altering their firing frequency, transneuronally change the number of postsynaptic sites. This prediction is now being tested in more complex parts of the nervous system.

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Visual response in barnacle photoreceptors is not initiated by transitions to and from metarhodopsin

ABSORPTION of a photon by the visual pigment of a photoreceptor results in a cascade of changes in the pigment molecule. At some time during the later stages of this cascade, a visual response appears, in the form of a change of the ionic conductance of the cell. In order to understand the mechanism of the process coupling the pigment changes to the modulation of the conductance, one should know from which state(s) or transition(s) of the pigment cascade the coupling process originates. In the vertebrate, it is commonly assumed^{1,2} that the fourth transition in the cascade, that from the state called metarhodopsin I to the state metarhodopsin II, is the predominant source of the coupling, mainly because this is the first transition involving a major molecular conformational change and the last temporally before the appearance of the visual response. Here we show that in the barnacle photoreceptor, transitions to and from the state called metarhodopsin (although its relation to vertebrate metarhodopsin is unclear) cannot be primarily involved in originating the coupling process, which must arise from earlier stages, not later than the second transition of the cascade.

In many invertebrate visual pigments, the metarhodopsin state is long lived and so can easily be photo-excited. We have shown that the barnacle pigment transition scheme contains return loops, so that most of the thermal transitions are activated in some degree by photoexcitation of both rhodopsin (Rh) and metarhodopsin (M) (see Fig. 1). By demonstrating that the shape of the late receptor potential (LRP) action spectrum is close to that of the photosensitivity spectrum of rhodopsin and is unaffected by shifts in the rhodopsin-metarhodopsin population ratio, we are able to exclude as major sources of the coupling to the LRP response all metarhodopsin-activated transitions and all states fed by these transitions. The only remaining candidates for the coupling are the phototransition from rhodopsin and its immediate thermal successor—the first rhodopsin photoproduct state.

Our previous analysis of the transition scheme of the barnacle photo-receptor visual pigment was based on early receptor potential (ERP) observations³. This scheme is shown in Fig. 1, omitting photo-transitions from the thermally unstable states since the intensities used in this experiment were always so low as to make them negligible. Figure 1 shows that photo-excitation of metarhodopsin strongly activates all of the transitions except Rh $\leftrightarrow$ B and B $\rightarrow$ C. If any of the metarhodopsin-activated transitions were substantially coupled to the LRP response, the action spectrum of the LRP should either be the metarhodopsin photosensitivity spectrum (for coupling from transitions M $\leftrightarrow$ E and E $\rightarrow$ F) or a weighted sum of the spectra of metarhodopsin and rhodopsin (transitions C $\rightarrow$ M, C $\rightarrow$ F, F $\rightarrow$ C and F $\rightarrow$ Rh). For each transition putatively coupled to the LRP, the LRP action spectrum may be predicted from the transition scheme and parameters shown in Fig. 1 and the initial population ratio of the rhodopsin and metarhodopsin states. This ratio depends on the wavelength of the light to which the preparation has been adapted and may either be calculated for this wavelength from the transition scheme

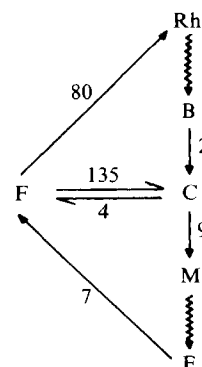


Fig. 1 The transition scheme of the barnacle visual pigment. The values are the thermal time constants of the transitions in ms at 20 °C. Rh, rhodopsin; M, metarhodopsin. Smooth arrows indicate thermal transitions, zigzag arrows phototransitions. The scheme and constants are those derived from early receptor potential observations³ except that phototransitions from unstable states are here omitted since their effects are small at the adapting light intensities used in the present experiment, and except for a small temperature correction (23 °C $\rightarrow$ 20 °C) of the thermal constants.

and parameters or may be directly measured using the ERP⁴. In this experiment we have measured the LRP action spectra following saturating adaption at two wavelengths which give the largest population difference; we have used ERP data to determine the rhodopsin-metarhodopsin population ratio in each case; and with those ratios we have calculated, for each putatively coupled transition, the expected LRP action spectrum. By comparing the positions of the observed LRP action spectra and the difference between them with these predictions, we are able to exclude all of the metarhodopsin-related transitions and states as dominant couplers. (LRP action spectra have previously been measured in the barnacle with various adaptations, but in conditions which do not permit determination of the pigment state during the measurements⁵.)

All measurements were intracellular recordings from excised lateral ocelli of *Balanus amphitrite* at 20 °C. Cells were adapted and stimulated by light from a quartz-iodide lamp passed through Balzers broad band (K2 or K3 for 'blue' and K6 for 'red') and narrow band (B 40) interference filters respectively.

In the barnacle, the rhodopsin and metarhodopsin spectra have peaks at about 532 and 495 nm respectively⁶⁻⁸. Red adaptation maximises the metarhodopsin population and blue the rhodopsin. According to ERP measurements, the rhodopsin:metarhodopsin ratios were about 80:20 and 5:95 following blue and red adaptation respectively.

The LRP action spectra following red and blue adaptation were measured by determining, at each wavelength, the intensity of a brief (30 or 300 ms) flash of light required to elicit a criterion small (3 mV or less) LRP response. This was done only when the sensitivity of the cell had reached (or nearly reached) a plateau, which sometimes took 2 or 3 h after the bright adapting illumination. The resulting action spectra, the wavelength dependences of the reciprocal of the amount of light needed to produce a criterion response at each wavelength, are displayed in Fig. 2. Both spectra are normalised by placing at 1 the peaks of smooth curves fitted to the points. (Observations on the ratio of the absolute sensitivities and the relation of this ratio to the rhodopsin population have been briefly reported^{9,10} and will be presented in detail elsewhere.)

Also shown in Fig. 2 for comparison are smooth curves matched to the ERP spectra^{6,7} (photometrically confirmed⁸) of rhodopsin (curve *a*) and metarhodopsin (curve *b*), together with a curve which is a 50:50 weighted sum of the two 'pure' curves. All three curves are also normalised to 1 at their peaks.

It is clear that both of the LRP spectra are, to a high confidence level, much closer to the rhodopsin ERP spectrum than is the intermediate curve; in other words, the metarhodopsin contribution to these spectra is appreciably less than 0.5. Furthermore, we estimate that the difference between the metarhodopsin contributions to the two LRP spectra is less than about 0.2 (much smaller scatter and possibility of systematic error; the bulk of the uncertainty in the ERP-LRP comparison arises from the ERP measurements).

For comparison with these experimental limits, we present in Table 1 the predicted contribution of the metarhodopsin spectrum to the LRP spectrum on the assumption that each of the transitions listed is the sole source of coupling to the LRP. It is clear that the only transitions not excluded by the experimental results are $Rh \leftrightarrow B$ and $B \rightarrow C$. Furthermore, since the transition rate from a particular state is proportional to the population of that state, all states but B are also excluded. The coupling to the LRP response must therefore arise predominantly from one or both of these transitions or from state B. Upper limits to the possible contributions from other states and transitions can be calculated from Table 1.

Having shown that metarhodopsin does not contribute to the LRP, we stress that it does have a physiological effect: excitation of metarhodopsin depresses or prevents the induction of prolonged depolarising afterpotentials¹¹. It may also influence the LRP at stimulus intensities higher than those used here¹¹.

Note that both of the transitions allowed as couplers are much faster (<3 ms) than the LRP latency. The coupling process must therefore involve a slow intermediate (non-pigment) process.

Fig. 2 Experimental and theoretical late receptor potential (LRP) action spectra. The points are the LRP sensitivities (reciprocals of numbers of photons needed to elicit criterion LRP responses) following saturating red (●) and blue (△) adaptation of the cells. Each point is the average of measurement from two cells. The curves are smooth fits (by eye) to the early receptor potential action spectra of rhodopsin (a) and metarhodopsin^{6,7} (b) (with small wavelength corrections from recalculations) as confirmed by photometry⁸. Curve (c) is a 50:50 sum of (a) and (b). All three curves as well as the experimental points are normalised to 1 at their peaks. Since red adaptation shifts most of the pigment into the metarhodopsin state, while even blue adaptation leaves some in that state, any substantial coupling of metarhodopsin-related transitions or states to the generation of the LRP should exhibit itself in a leftward shift (from the rhodopsin curve) of the triangles and, much more strongly, the circles. The smallness of any such shifts is interpreted in the text as showing that, of the states and transitions of the scheme of Fig. 1, only the transitions $Rh \leftrightarrow B$ and $B \rightarrow C$ and the state B can be substantially coupled to the generation of the LRP.

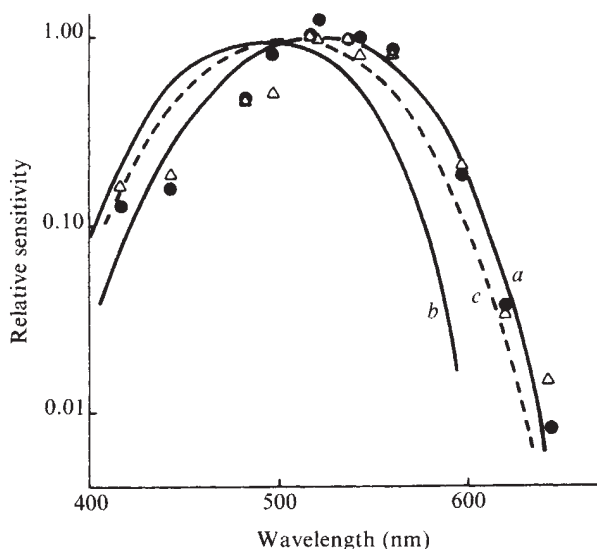


Table 1 Calculated contributions of the metarhodopsin spectrum to the LRP spectrum for various transitions possibly coupled to the LRP

Transition	Fraction of photoexcitations going through transition from		Metarhodopsin contribution to LRP spectrum following adaptation by		
	Rh	M	Blue	Red	Δ
$Rh \leftrightarrow B$; $B \rightarrow C$	1	0	0	0	0
$C \rightarrow M$; $C \rightarrow F$	0.4	0.15	0.92	0.13	0.79
$F \rightarrow C$; $F \rightarrow A$	0.6	0.85	0.98	0.36	0.62
$M \leftrightarrow E$; $E \rightarrow F$	0	1	1	1	0
Experimental	—	—	<0.5	<0.5	<0.2

The first two numerical columns give the fractions of rhodopsin and metarhodopsin photoexcitations (respectively) which pass through the transitions indicated, as calculated from Fig. 1. The metarhodopsin contributions to the LRP spectrum after blue and red adaptation are presented in the next two columns. These are calculated from the first two columns as follows: the metarhodopsin fraction divided by the sum of the two fractions is multiplied by the relative metarhodopsin populations after blue and red adaptation (0.2 and 0.95) and by the ratio⁸ of the peak photosensitivities of metarhodopsin and rhodopsin (1.6). The final column gives the differences between the values of the two preceding columns and indicates the expected spectrum shift resulting from adaptation. The final row gives the experimental limits.

An alternative approach to the location of the transduction coupling would be an examination of the dependence on rhodopsin population of the LRP sensitivity at a particular wavelength or band of wavelengths. If the dependence is linear at wavelengths at which metarhodopsin has an appreciable sensitivity, any contribution from metarhodopsin-excited transitions is excluded (except for unlikely accidental cancellations). Such a linear dependence was indeed found in the octopus, fly and moth¹². The detailed pigment schemes are, however, not known in these animals. Nonlinear dependences have been observed in the barnacle^{9,10} and in the blowfly¹³ but the spectral measurements described above exclude a metarhodopsin contribution as the source of the nonlinearity in the barnacle and the absence of such measurements allows no conclusion for the blowfly.

The various pigment states in the barnacle have not been studied biochemically; our results provide considerable incentive for such a study. If the transition to metarhodopsin is shown to be the first in the chain to involve a major conformational change, as in the vertebrate, that change will have been shown not to play a vital part in the transduction process in this system.

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Note added in proof: Strong and Lisman¹⁴ have shown that metarhodopsin also does not contribute to light adaptation of this photoreceptor.

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Possible role of cyclic nucleotides in regulation of noradrenaline release from rat pineal through presynaptic adrenoceptors

PRESYNAPTIC α -adrenoceptors on noradrenergic nerve endings have been shown to mediate a negative feedback mechanism which leads to inhibition of transmitter release during depolarisation induced by nerve stimulation or potassium^{1–3}. On the other hand, it is known that in some tissues activation of presynaptic β -adrenoceptors both *in vitro* and *in vivo* leads to an increase in transmitter release^{4–8}. The α -adrenoceptors seem to operate by restricting the calcium available for the excitation–secretion coupling, and the facilitating effect of β -agonists on transmitter release seems to be mediated through an increase in the levels of cyclic AMP in noradrenergic nerve endings^{7,8}. Recent reports^{9,10} have provided electrophysiological evidence for a prejunctional role of cyclic nucleotides in neurotransmission. In the rat pineal gland a calcium-dependent presynaptic mechanism for the generation of cyclic GMP has been reported which might be linked to an α -adrenergic-like receptor¹¹. In addition, there is evidence in this gland for an α -adrenergic presynaptic mechanism regulating noradrenaline release elicited by potassium¹². Consequently, we decided to examine and report here our finding of a role of cyclic nucleotides in noradrenaline release from the rat pineal gland and the possible involvement of these substances in the regulatory mechanisms mediated through the α and β presynaptic adrenoceptors.

For transmitter release studies we used a previously described technique¹³. Male rats (160–200 g) were killed by decapitation and their pineal gland removed immediately. For each experiment four pineals were placed in an open lucite cylinder with a piece of nylon mesh fitted to the bottom. The system was placed in a beaker containing 5 ml of Krebs bicarbonate solution at 37°C and was bubbled with a 95% O₂:5% CO₂ mixture. The endogenous noradrenaline stores were labelled *in vitro* by incubating the pineals with 0.5 μ M ($\pm$)-7-³H-noradrenaline for 30 min. When the spontaneous efflux of radioactivity had levelled off 50 min later, ³H-neurotransmitter release was elicited either by a one-minute exposure to media containing 60 mM KCl or by a five-minute exposure to 3 μ M tyramine (Fig. 1). During depolarisation with 60 mM KCl an equimolar reduction of NaCl was carried out to maintain the osmolarity of the medium.

In the controls, the fraction of the total tissue radioactivity released by the first exposure to potassium was 17.13 ± 1.05 ($\times 10^{-3}$) ($n=74$) and the ratio obtained between the two consecutive stimulation periods (S_2/S_1) was 1.15 ± 0.10 ($n=10$) (Table 1). In these experimental conditions, ³H-noradrenaline release by potassium was found to be entirely calcium dependent¹². Denervated pineal glands (seven days after bilateral superior cervical ganglionectomy) failed to retain ³H-noradrenaline and did not release tritium when exposed to 60 mM potassium.

Exposure to oxymetazoline (10 μ M), an α agonist that has been reported to be more selective for presynaptic α -receptors³, produced a significant inhibition of ³H-noradrenaline release. On the other hand, yohimbine (10 μ M), a selective

presynaptic α -blocking agent³, increased transmitter overflow more than twofold (Table 1). When ³H-noradrenaline release was elicited by exposure to 3 μ M tyramine, the fraction of total tissue radioactivity released by S_1 was 38.15 ± 1.48 ($\times 10^{-3}$) ($n=42$) and the ratio between two consecutive stimulation periods was 1.23 ± 0.10 , $n=8$ (Table 2). The effects of oxymetazoline and yohimbine on presynaptic α -receptors were selective for the release induced by potassium, as these drugs did not modify ³H-neurotransmitter release elicited by exposure to tyramine (compare Tables 1 and 2).

To investigate the presence of presynaptic β -receptors in the noradrenergic nerve endings of the rat pineal gland, we tested the agonists isoprenaline (14 nM) and terbutaline (80 nM). Both drugs significantly enhanced ³H-noradrenaline release

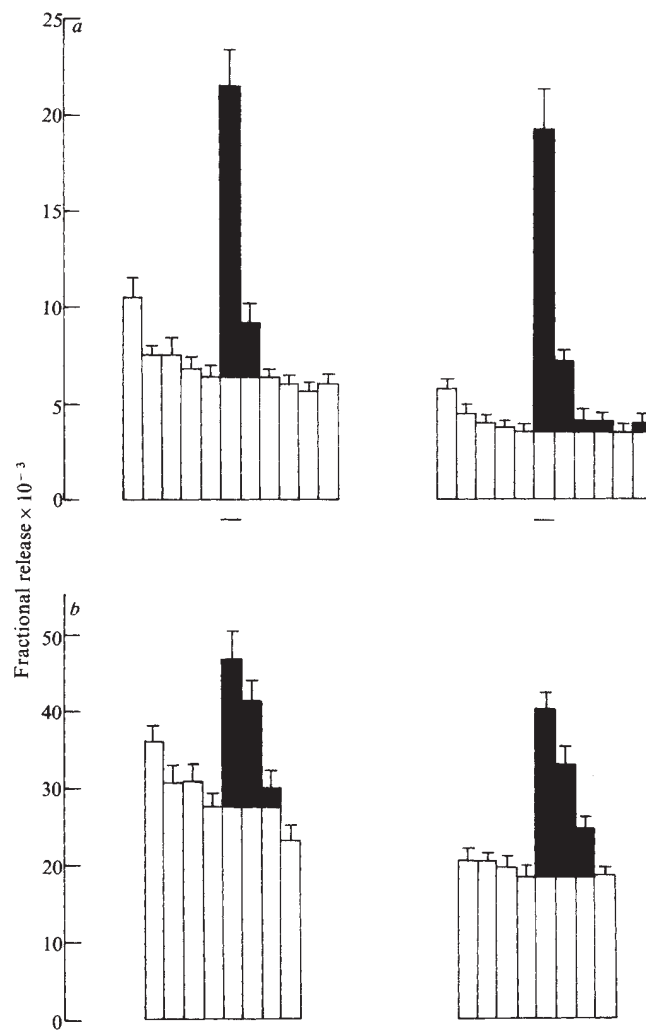


Fig. 1 Release of radioactivity from the rat pineal gland prelabelled with ³H-noradrenaline during exposure to potassium or tyramine. Ordinates: fractional release ($\times 10^{-3}$): fraction of the total tissue radioactivity released per sample. *a*, Release of ³H-noradrenaline evoked by 60 mM K⁺ ($n=10$). The interval between two consecutive potassium stimulations was 35 min. One-minute samples were collected. Tissue content at the end of the experiment was 252.2 ± 14.8 nCi per four pineals ($n=10$). *b*, Release of ³H-noradrenaline evoked by tyramine 3 μ M ($n=8$). The interval between two exposures to tyramine was 40 min. Five-minute samples were collected. Tissue content at the end of the experiment was 239.1 ± 25.8 nCi per four pineals ($n=8$). Unshaded columns, spontaneous outflow of total radioactivity collected in each sample (1 min in *a* and 5 min in *b*). Shaded columns, increase in radioactivity induced by exposure to potassium or tyramine. The horizontal bars under the abscissae indicate the period of exposure to potassium (*a*) or tyramine (*b*). Mean values $\pm$ s.e.m. are shown; n , number of experiments per group.

Table 1 Effects of α -adrenoceptor activation and blockade on ^3H -transmitter release elicited by potassium in the rat pineal

Experimental group	n	Fractional release ($\times 10^{-3}$)		Ratio S_2/S_1
		S_1	S_2	
Controls	10	16.87 ± 2.49	18.03 ± 2.73	1.15 ± 0.10
Oxymetazoline $10 \mu\text{M}$	6	12.51 ± 2.63	$7.51 \pm 1.56^*$	$0.60 \pm 0.03^*$
Yohimbine $10 \mu\text{M}$	5	13.89 ± 1.72	$33.20 \pm 5.78^\dagger$	$2.32 \pm 0.27^\ddagger$

Fraction of the total tissue radioactivity released by a 60-s exposure to 60 mM K^+ . S_1 corresponds to the first exposure to K^+ and S_2 to the second, obtained 35 min after S_1 . All drugs were added to the incubation medium 20 min before S_2 . Mean values $\pm$ s.e.m. are shown.

* $P < 0.001$ when compared against the control group.

$^\dagger P < 0.05$.

$^\ddagger P < 0.005$.

induced by potassium. The ratios S_2/S_1 were 1.86 ± 0.22 ($n = 6$; $P < 0.02$) and 2.14 ± 0.29 ($n = 4$; $P < 0.01$), respectively. In these conditions, exposure to ($\pm$)propranolol ($0.1 \mu\text{M}$) alone did not significantly affect ^3H -transmitter release induced by potassium ($S_2/S_1 = 1.19 \pm 0.11$, $n = 6$). However, this concentration of the β -blocking agent antagonised the facilitating effect of isoprenaline on transmitter overflow ($S_2/S_1 = 0.95 \pm 0.15$; $n = 4$), supporting the view that presynaptic β -adrenoceptors are present in the rat pineal gland.

Several nucleotide analogues have been described which differ in their metabolic stability and in their ability to cross cell membranes. Thus, we tested N^6 -2'- O -dibutyryl-adenosine-3',5'-cyclic monophosphoric acid (dibutyryl cyclic AMP) and N^2 -2'- O -dibutyryl-guanosine-3',5'-cyclic monophosphoric acid (dibutyryl cyclic GMP). Exposure to dibutyryl cyclic AMP during the second potassium stimulation period increased ^3H -transmitter overflow in a concentration-dependent manner (Fig. 2a), but not that induced by $3 \mu\text{M}$ tyramine (Fig. 2a). In the presence of 0.1 mM dibutyryl cyclic AMP there was a small but statistically significant reduction in the release of ^3H -noradrenaline by tyramine. This increase in potassium-evoked transmitter release observed with dibutyryl cyclic AMP supports the hypothesis that presynaptic β -adrenoceptors might be linked to adenylate cyclase activation in noradrenergic nerve endings⁸.

Addition of dibutyryl cyclic GMP 20 min before the second potassium stimulation produced an inhibition of ^3H -neurotransmitter release in a concentration-dependent manner (Fig. 2b). In conditions in which the α -presynaptic adrenoceptors were blocked by $10 \mu\text{M}$ yohimbine, potassium-evoked release of ^3H -noradrenaline was also significantly reduced by 0.5 mM dibutyryl cyclic GMP. In contrast with the results observed for potassium, dibutyryl cyclic GMP did not modify transmitter overflow evoked by exposure to $3 \mu\text{M}$ tyramine (Fig. 2b). It is of interest that the reduction in potassium-evoked noradrenaline overflow observed in the presence of dibutyryl cyclic GMP was not due to an increase in neuronal uptake of noradrenaline. In our experiments 0.5 mM dibutyryl cyclic GMP did not affect the neuronal uptake of ^3H -noradrenaline in conditions in which $1 \mu\text{M}$ cocaine reduced the neuronal accumulation of ^3H -neurotransmitter from $10.37 \pm 0.68 \text{ nCi}$ per pineal ($n = 7$) to $2.68 \pm 0.17 \text{ nCi}$ per pineal ($n = 6$; $P < 0.001$). It has been reported that α -adrenergic stimulating agents specifically increase cyclic GMP levels in cultured rat pineals. The increase in cyclic GMP levels elicited by ($-$)noradrenaline or potassium depolarisation was shown to be calcium dependent and was markedly reduced in denervated pineals, indicating a possible presynaptic site of action¹¹. The reduction in potassium-evoked transmitter release which was observed in the presence of dibutyryl cyclic GMP is compatible with the view that cyclic

GMP may be a link in the chain of events following activation of presynaptic α -adrenoceptors and leading to a decrease in the release of noradrenaline from the rat pineal gland.

To test further the hypothesis that the modulation of noradrenaline release mediated by presynaptic α - and β -adrenoceptors involves changes in the levels of neuronal cyclic nucleotides, experiments were carried out in the presence of selective cyclic AMP- and cyclic GMP-phosphodiesterase inhibitors. The selective inhibitor of the cyclic AMP-specific phosphodiesterase used was 4-(3-cyclopentyl-4-methoxyphenyl)-2-pyrrolidone (ZK 62711) (ref. 14). This agent in a concentration of $10 \mu\text{M}$ was added before S_2 and did not modify the potassium-evoked transmitter release ($S_2/S_1: 1.19 \pm 0.31$, $n = 6$). However, in experimental conditions in which the α -presynaptic receptors were blocked during S_1 and S_2 by $10 \mu\text{M}$ yohimbine, exposure to $10 \mu\text{M}$ ZK 62711, during S_2 significantly enhanced the release of ^3H -noradrenaline induced by potassium ($S_2/S_1 = 2.23 \pm 0.31$, $n = 5$, $P < 0.01$ when compared with the corresponding controls in the presence of yohimbine, $S_2/S_1 = 1.11 \pm 0.10$, $n = 6$).

The selective inhibitor of the cyclic GMP-specific phosphodiesterase was 2- O -propoxyphenyl-8-azapurin-6-one (MB 22948) (ref. 15). When this drug was added in a concentration of $10 \mu\text{M}$ before S_2 there was a significant inhibition of the potassium-evoked transmitter release ($S_2/S_1: 0.78 \pm 0.10$, $n = 6$, $P < 0.05$ when compared with the corresponding controls, $S_2/S_1: 1.15 \pm 0.10$, $n = 10$). A similar decrease in ^3H -noradrenaline release by MB 22948 was obtained when the β -presynaptic receptors were blocked by ($\pm$)propranolol. This β blocker, added alone in a concentration of $0.1 \mu\text{M}$ before the second stimulation, did not modify the potassium-evoked release ($S_2/S_1: 1.19 \pm 0.11$, $n = 6$). However, a significant decrease in ^3H -noradrenaline release was obtained when $0.1 \mu\text{M}$ ($\pm$)propranolol plus $10 \mu\text{M}$ MB 22948 were added before S_2 ($S_2/S_1: 0.68 \pm 0.13$, $n = 5$, $P < 0.05$ when compared with the corresponding control).

The reduction in the potassium-evoked transmitter release which was observed in the presence of dibutyryl cyclic GMP and during exposure to the inhibitor of the cyclic GMP-specific

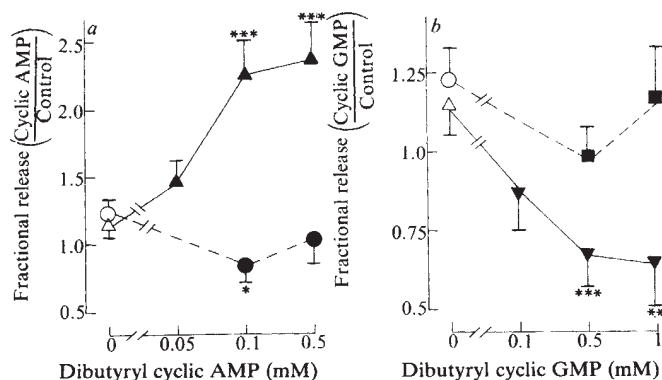


Fig. 2 Comparative effects of cyclic nucleotides on potassium and tyramine-induced release of ^3H -noradrenaline from the rat pineal gland. Ordinates: ratio of fractional release between the second and the first stimulation carried out within the same experiment after exposure to potassium (60 mM) or tyramine ($3 \mu\text{M}$). Dibutyryl cyclic AMP (a) or dibutyryl cyclic GMP (b) were added to the medium 20 min before the second stimulation and kept until the end of the experiment. Only one concentration of the drug was tested in each experiment. The open symbols correspond to the ratio of the controls when ^3H -noradrenaline release was induced by potassium (Δ) or tyramine ($\circ$). Added before the second potassium exposure: dibutyryl cyclic AMP ($\blacktriangle$) or dibutyryl cyclic GMP ($\blacktriangledown$). Added before the second tyramine exposure: dibutyryl cyclic AMP ($\bullet$) or dibutyryl cyclic GMP ($\blacksquare$). Mean values $\pm$ s.e.m. of at least five experiments per group are shown. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$ when compared against the corresponding control.

Table 2 Effects of α -adrenoceptor activation and blockade on ^3H -transmitter release elicited by tyramine in the rat pineal

Experimental group	n	Fractional release ($\times 10^{-3}$)		Ratio S_2/S_1
		S_1	S_2	
Control	8	36.39 ± 3.13	43.21 ± 2.57	1.23 ± 0.10
Oxymetazoline $10 \mu\text{M}$	5	34.65 ± 2.52	45.55 ± 5.09	1.33 ± 0.17
Yohimbine $10 \mu\text{M}$	5	29.77 ± 4.99	41.71 ± 6.64	1.42 ± 0.08

Fraction of the total tissue radioactivity released by a 5-min exposure to tyramine $3 \mu\text{M}$. S_1 corresponds to the first exposure and S_2 to the second, obtained 40 min after S_1 . All drugs were added to the incubation medium 20 min before S_2 . Mean values $\pm$ s.e.m. are shown.

phosphodiesterase supports the view that cyclic GMP may be a link in the chain of events following activation of presynaptic α -adrenoceptors and leading to a decrease in the stimulation-evoked release of noradrenaline from the rat pineal gland.

The finding that the selective cyclic AMP-phosphodiesterase inhibitor ZK 62711 increased stimulation-evoked release only when the presynaptic α -adrenoceptors were blocked with yohimbine can be explained as follows. During normal transmitter release neuronal cyclic GMP levels are increased as a result of activation of presynaptic α -adrenoceptors. As already described for other tissues cyclic GMP is linked to physiological events which are opposite to those of cyclic AMP (ref. 16). Consequently, it is possible that the facilitatory effects of the selective inhibition of cyclic AMP-phosphodiesterase on transmitter release can only be observed when the simultaneous increase in neuronal cyclic GMP levels is prevented by blockade of presynaptic α -adrenoceptors.

Tyramine elicits noradrenaline release by displacing the transmitter from vesicular binding sites. In contrast to potassium, tyramine-evoked release of ^3H -transmitter is not calcium dependent, and is not regulated through α -adrenergic presynaptic receptors¹⁷. The failure of dibutyl cyclic AMP to enhance and of dibutyl cyclic GMP to reduce tyramine-evoked noradrenaline release significantly would indicate that these nucleotides are only involved with changes in noradrenaline release induced through calcium-dependent processes (that is, nerve stimulation or potassium-evoked release).

In summary, our results are compatible with the existence in the rat pineal gland of α - and β -presynaptic adrenoceptors regulating noradrenaline release. In addition, it seems that an increase in the neuronal levels of cyclic GMP is linked to the events triggered by the activation of the inhibitory presynaptic α -adrenoceptors. The question remains as to whether cyclic GMP subserves a similar role in regulating noradrenaline release in other tissues. However, the results obtained with cyclic AMP and the specific cyclic-AMP-phosphodiesterase inhibitor further support the hypothesis that this cyclic nucleotide is linked to the positive feedback mechanism mediated by presynaptic β -adrenoceptors⁸.

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Light-induced calcium release in a photosensitive vertebrate smooth muscle

THE involvement of calcium in the transduction process in vertebrate photoreceptors, in particular rods, has been postulated by Yoshikami and Hagins¹, who suggest that this cation is sequestered in the membranous disks of the outer segment and released on stimulation by light. Recently, evidence supporting this hypothesis was obtained by Fishman *et al.*² who, using an electron microscopic technique^{3–5} for the localisation of calcium, showed precipitate within the disks of frog rod outer segments. However, they did not explore the possibility of a light-induced decrease of calcium within the disks, which would have further supported the hypothesis. We report here evidence for the involvement of calcium in the phototransduction process in a unique vertebrate photoreceptive system.

It has been known since the mid-nineteenth century that irises of many animals are endogenously photosensitive and autonomously constrict on stimulation by light, even when completely excised from the body and from other ocular structures^{6–8}. Much indirect evidence has indicated that the smooth muscle cells of the sphincter pupillae are directly photosensitive^{9–12}. Bito and Turansky¹², studying the iris of the golden hamster, which responds directly to both light stimulation and parasympathomimetic drugs such as carbachol, investigated the effects of decreased calcium ion concentration in the external medium. They found that merely eliminating calcium chloride from the solution blocked the cholinergic(carbachol) response, but did not affect the light response. Addition of 10^{-3}M EGTA to the calcium-free solution did, however, block the light-induced pupillary constriction. These data suggest that carbachol and light use different calcium pools, the pool of the former being loosely bound (or extracellular) and that of the latter being tightly bound somewhere in the cell. Nevertheless, these two pools probably ultimately affect the same contractile apparatus.

We used a technique similar to that of Fishman *et al.*² to localise calcium at the electron microscopic level in the hope of locating a light-sensitive pool of calcium, that is, one that is present in dark-adapted irises, absent or decreased in light-adapted irises, and unaffected by carbachol.

For these experiments, eyes were enucleated from Syrian hamsters that had been adapted to room light and then anaesthetised with pentobarbital sodium. The eyes were placed in Petri dishes filled with Krebs's solution (118.4 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl_2 , 25.0 mM NaHCO_3 , 1.2 mM MgSO_4 , 1.2 mM KH_2PO_4 and 11.1 mM glucose) and the scleras and corneas were cut. Light- or dark-adaptation of an iris before fixation involved placing it either under a bright light (8 cm from a microscope illuminator (American Optical 651)) or into darkness for 30 s. This length of time was chosen because

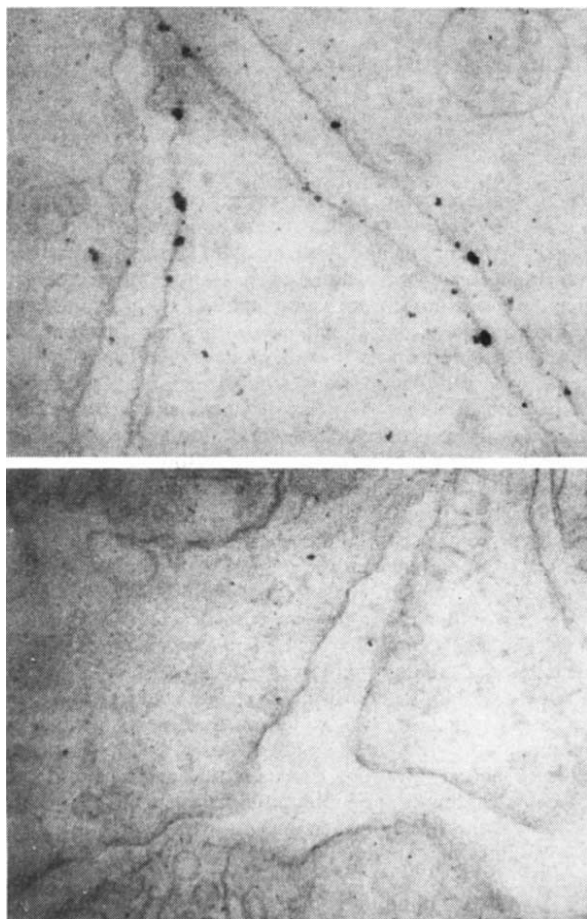


Fig. 1 Comparison of irises, 30-s dark-adapted (top) and light-adapted (bottom). Note that deposits tend to be localised on sarcolemma in dark-adapted iris but absent from light-adapted iris. Magnification: top, $\times 67,000$; bottom, $\times 87,500$.

light-induced constriction is then near its maximum¹². The fixation procedure involved placing the iris in 2% potassium pyroantimonate and 1% osmium tetroxide in distilled water, at a pH of 9.2 (the pH was adjusted using 0.1 N acetic acid) for approximately 80 min. Fixation was followed by rinsing in distilled water, dehydration in graded acetones, and embedding in Spurr's¹³ medium. The entire sequence was done at room temperature and in all cases the fixation step was carried out in total darkness. Carbachol experiments consisted of placing an iris in Krebs's solution with 10^{-8} M carbachol, in the dark, for 30 s before fixation. This concentration was chosen because it produced pupillary constriction equivalent to that produced by light (personal observation). The carbachol experiments were done not only to test the effects of this drug on a light-activated calcium pool, but also to ensure that any putative light-induced effects were not merely a result of the contractile state of the sphincter cells. Unstained thin sections in a radial orientation (providing approximate cross-section of the muscle cells) were examined in a Philips 300 electron microscope using an accelerating voltage of 40 kV.

Figure 1 shows sections from a pair of irises from the same hamster, one being dark-adapted (top) and the other light-adapted (bottom). The fixation procedures were carried out simultaneously and with solutions from the same stocks. There are discrete deposits associated with the sarcolemma of the sphincter smooth muscle cells of the dark-adapted iris. In contrast, these deposits are absent from the cells in the light-adapted iris. Similar results were obtained in many other pairs of light- and dark-adapted irises, although any attempt to

quantify the deposits either in number or size has not been possible due to the great variability among preparations. Preliminary results using this technique on photosensitive irises of frogs (*Rana pipiens*) have also shown this phenomenon.

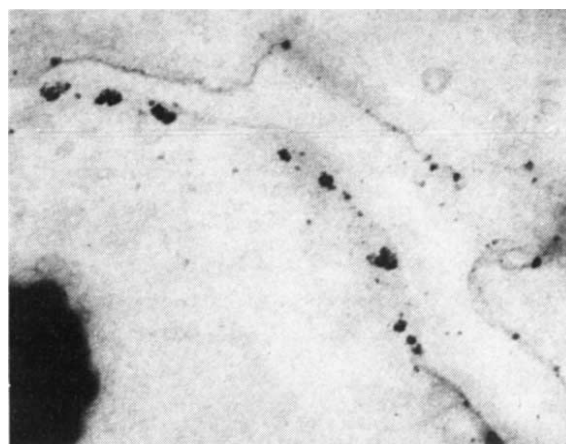
Figure 2 shows a section from an iris in which dark-adaptation occurred in the presence of 10^{-8} M carbachol. The presence of the deposits, which were never seen in light-adapted irises, indicates that the light-influenced calcium is not mobilised by carbachol. Other experiments using 10^{-7} M or 10^{-6} M carbachol gave similar results.

To determine whether deposits are indeed calcium precipitates, pieces of dark-adapted tissue were placed in 1 mM EGTA in distilled water for approximately 25 min after the fixation step, but before dehydration. This tissue was then compared with another piece of the same dark-adapted iris which had not undergone the EGTA treatment (Fig. 3). Deposits are noted in the untreated tissue but are absent from the treated tissue, thus strongly suggesting that the precipitate does consist of calcium salts.

As the deposits are removed by EGTA, they are almost certainly precipitates of a divalent cation¹⁴. The only likely alternative to calcium, in physiological conditions, is magnesium. As removal of external magnesium ions has no effect on the light response of frog irises (personal observation), and as chelating calcium ions in the presence of excess magnesium abolishes the light response of hamster irises¹², magnesium is probably not involved in this phenomenon. Furthermore, preliminary experiments on frog irises have shown that similar concentrations (10^{-3} M) of EGTA and EDTA are required to remove the deposits. Nakas *et al.*¹⁵ point out that if magnesium is the ionic species involved, EGTA and EDTA should affect the deposits in concentrations differing by approximately five orders of magnitude, whereas in the case of calcium approximately equal concentrations of the two chelators should be required. As this occurs in the frog iris, the presence of calcium is strongly indicated.

These results support the hypothesis that the smooth muscle cells of the pupillary sphincter have a dual contractile and photoreceptive function. More importantly, however, these experiments indicate light-induced calcium movement in a vertebrate photoreceptive system. The fact that the sphincter pupillae is embryologically derived from the same neuroectoderm that gives rise to the retina¹⁶, and the recent demonstration by Blaustein and Dewey¹⁷ of the presence of rhodopsin in the sarcolemma of frog pupillary sphincter cells suggest that the chain of events of phototransduction in vertebrate rods and cones is similar to that in the sphincter cells of the photosensitive iris and thus support the hypothesis of Yoshikami and Hagins¹. As this iris preparation is easily accessible, and its

Fig. 2 Iris dark-adapted in presence of 10^{-8} M carbachol. Deposits are noted on sarcolemma. Magnification: $\times 87,500$.



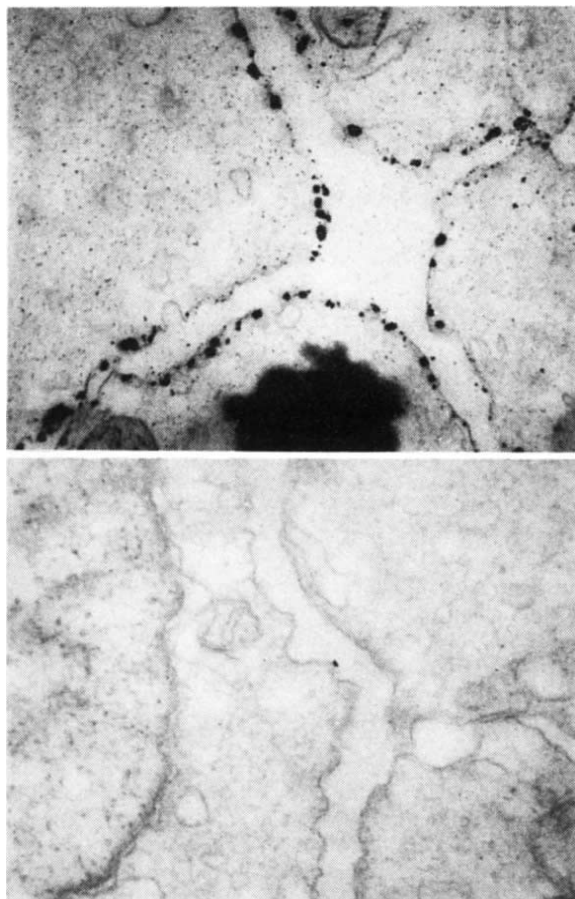


Fig. 3 Comparison of two sections from the same dark-adapted iris. Top, normal fixation; bottom, treated with 1 mM EGTA after fixation. Magnification: top, $\times 54,000$; bottom, $\times 67,000$.

responses stable and easily monitored (for example, using muscle tension and sphincter length changes), it may be useful for studying the processes involved in the transduction of light energy into neural signals.

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Thick-filament-linked calcium regulation in vertebrate striated muscle

CALCIUM is the trigger initiating muscular contraction, and relaxation occurs on its removal. As contraction is caused by the cyclic attachment and detachment of myosin-crossbridges of the thick filament with actin of the thin filament, calcium, in principle, could modify muscular activity by affecting regulatory systems on either the thick or the thin filaments. It is generally accepted that a thin-filament-linked Ca^{2+} -regulatory system, involving troponin and tropomyosin, is responsible, at least in part, for the on-off switch governing contraction in vertebrate striated muscle^{1,2}. Several studies also suggest the possibility of a simultaneously operating Ca^{2+} -regulatory system on the thick filaments, presumably involving the myosin molecules³⁻⁶. However, the actin-activated Mg-ATPase of purified vertebrate striated muscle myosin has not been shown to be Ca^{2+} dependent, and hence direct evidence for a thick-filament-linked regulatory system is lacking⁶. Nonetheless, it is debatable whether the latter evidence precludes the existence of a thick-filament-linked system. The effect may simply result from an essential component of the thick filament being lost or denatured during standard myosin purification procedures. Alternatively, actomyosin which is routinely formed from repolymerised pure proteins may not assume the proper quaternary structure allowing for expression of myosin's Ca^{2+} sensitivity. In view of the ambiguities inherent in these studies, we adopted another approach and report here our attempts to test for thick-filament-linked Ca^{2+} regulation in actomyosin preparations in which we had removed troponin-tropomyosin from the thin filaments. The Ca^{2+} sensitivity of intact thick filaments could then be measured with both the residual and added pure actin. Our results do suggest the presence of a thick-filament-linked Ca^{2+} -regulatory system.

The procedure used was developed by Perry *et al.*⁷ Myofibrils are extensively washed in low ionic strength buffers, causing the troponin and tropomyosin to dissociate from thin filaments but leaving the thick filaments unaltered⁸. In the present study the method was modified as follows. Rabbit myofibrils from longissimus dorsi muscles (75 g), prepared as previously⁶, were resuspended in 1 l 1 mM dithiothreitol (or dithioerythritol), 0.1 mM NaN_3 and 5 mM Tris buffer (pH 8.0) and homogenised for 1 min in a Sorvall Omnimixer. The homogenate was sedimented at 25,000g for 10 min, the pellets resuspended in the above solution, and the sedimentation and resuspension step repeated three more times. The final pellets were homogenised in 1 l of the above solution and dialysed overnight against 8 l of the same solution. On each of three successive days, homogenisation, sedimentation and resuspension, and dialysis steps were repeated using 0.1 mM NaN_3 , 5 mM Tris (pH 8.0) (without use of sulphhydryl reagents). The preparation was homogenised once more before ATPase assays were carried out.

SDS-polyacrylamide gel electrophoresis of this low ionic strength washed myofibrillar preparation shows the presence of actin and the myosin heavy and light chains (Fig. 1, gel a). In contrast, tropomyosin is present in very low concentration (compare Fig. 1, gels a and b). The persistence of trace amounts of tropomyosin does not seem to be affected by more extensive low ionic strength treatment. Another polyacrylamide gel system was used to test for the presence of the troponin complex, as some of its components co-migrate with myosin light chains on SDS gels. Alkaline-urea gels, which can be used to detect the troponin-C subunit⁹ (Fig. 1, gels c and d), show that troponin-C and presumably whole troponin are absent in the preparation (Fig. 1, gels e and f). These results, therefore, support earlier work⁷ and show that the washed myofibrils are depleted of troponin and tropomyosin.

Table 1 ATPase activities of low ionic strength washed myofibrils

Sample	[NaCl](mM)	Mg-ATPase*		Calcium sensitivity	Actin activation
		+ EGTA	+ Calcium		
a, 2 mg myofibrils	30	0.42	0.39	—	—
b, 2 mg myofibrils	80	0.26	0.28	7%	—
c, 2 mg myofibrils	100	0.14	0.23	41% (40–42)	—
d, 2 mg myofibrils	120	0.048	0.11	57% (41–67)	—
e, 2 mg myofibrils + 2 mg pure actin	120	0.075	0.28	73% (68–75)	2.5 (1.7–4.0)
f, ATPase e-d	120	0.027	0.16	83% (76–88)	

Results are averages obtained from four different low ionic strength washed myofibril preparations. ATPase assays were carried out using the pH stat method¹² in 10 ml 0.7 mM ATP, 1 mM MgCl₂, pH 7.5, 25°C. The [NaCl] was varied as indicated. Ca²⁺ sensitivity was determined by comparing ATPase activity in the presence of 0.1 mM EGTA alone and with 0.1 mM EGTA containing 0.2 mM CaCl₂ by using the following relationship:

$$\text{Ca}^{2+} \text{ sensitivity} = \frac{\text{ATPase}_{\text{Ca}} - \text{ATPase}_{\text{EGTA}}}{\text{ATPase}_{\text{Ca}}} \times 100$$

Activation of the ATPase_{Ca} by actin was calculated by the following relationship:

$$\text{Actin activation} = \frac{\text{ATPase}_{\text{Ca}} \text{ with added actin}}{\text{ATPase}_{\text{Ca}} \text{ without added actin}}$$

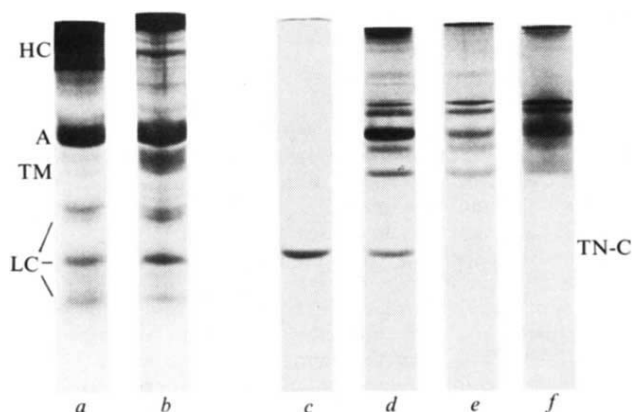
The extra ATPase rate stimulated by the added actin was computed from the difference in activities in the presence and absence of the added actin (line f). Values in parentheses indicate the range of values observed.

* μmol of ATP split per min per mg of myofibrils.

ATPase assays on the washed myofibrillar preparation show a lack of Ca²⁺ sensitivity at monovalent ion concentrations lower than 100 mM (Table 1a and b), but at 120 mM NaCl Ca²⁺ sensitivity is observed (Table 1d). The ATPase of the preparation is activated, at 120 mM NaCl, by additional pure actin and the resultant activity remains Ca²⁺ sensitive (Table 1e). In fact, the fraction of the ATPase activated by the added pure actin is more Ca²⁺ sensitive than the initial ATPase (Table 1f), indicating that the Ca²⁺ dependency is not artefactual.

These results show that myosin contained in this preparation can be activated by pure actin in a Ca²⁺-dependent fashion. As the actin is free of troponin-tropomyosin, the Ca²⁺ dependency seems to arise from the myosin-containing thick filaments themselves, and thus these data support other evidence suggesting the presence of a thick-filament-linked regulatory system^{3–6}.

Fig. 1 Polyacrylamide gel electrophoresis of various rabbit muscle preparations stained in Coomassie brilliant blue dye. a, b, SDS gels¹¹ (10% acrylamide): a, low ionic strength washed myofibrils, 75 μg ; b, unwashed myofibrils, 75 μg . c–f, alkaline-urea gels⁹ (8% acrylamide): c, purified troponin-C, 5 μg ; d, unwashed myofibrils, 75 μg ; e, f, low ionic strength washed myofibrils, 75 μg , 240 μg . Samples run on alkaline-urea gels contained 5 mM EGTA. Abbreviations: A, actin; TM, tropomyosin; TN-C, troponin-C; HC, myosin heavy chain; LC, myosin light chains.



In a previous study⁶ to test the effect of excess pure actin on troponin-tropomyosin-containing rabbit myofibrils (the competitive actin-binding assay), we pointed out that a Ca²⁺ dependence is observed at physiological monovalent ion concentrations but not low ionic concentrations. We concluded that the binding of the myosin to actin, at low ionic concentrations, increases sufficiently to override the Ca²⁺ dependency. We have confirmed this observation here with a different type of preparation.

A potential role of a thick-filament-linked system in the overall scheme of Ca²⁺ regulation remains to be evaluated. One possible advantage for a dual system of Ca²⁺ control, involving the thick and the thin filaments, could be the resultant enhancement of the Ca²⁺ dependency. This would result from both the thin and the thick filaments, which must interact for ATP hydrolysis and hence for contraction, being inhibited in the absence of Ca²⁺. Alternatively, the troponin-tropomyosin based thin-filament-linked regulation may actually be a sufficiently effective on-off switch; the thick-filament-linked system may fulfill another role, varying the degree of muscular contraction by adjusting the extent of the actin-myosin interaction once the thin filament is switched on¹⁰. As the apparent binding constant of Ca²⁺ to vertebrate myosin has been reported to be weaker than that for troponin¹³, this modulation may depend on the exact Ca²⁺ concentration in the sarcoplasm. These and other hypotheses await experimental verification.

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Immunohistochemical evidence for myosin polymorphism in the chicken heart

MULTIPLE forms of myosin are known to be present in vertebrate striated muscle. On the basis of the light-chain pattern and of other structural and enzymatic features, three distinct types of myosin have been identified in fast-skeletal, slow-skeletal and cardiac muscles of mammals and birds¹⁻⁵. Although skeletal muscle fibre heterogeneity has been generally recognised in these comparative studies, it has always been tacitly assumed that cardiac muscle consists of a homogeneous cell population in terms of myosin. However, the heart comprises muscle cells which differ in structure and function, such as atrial cells, ventricular cells and the specialised cells of the conducting system. We report here immunohistochemical evidence for the existence of distinct myosins in these different muscle cell types in the chicken heart.

Specific antisera were prepared against column-purified myosins from the slow anterior latissimus dorsi muscle, the fast pectoralis muscle and the heart ventricle of adult chicken. Myosins were isolated following the method described by Barany and Close⁶, using ion-exchange chromatography for final purification⁷, and proved to be essentially free of contaminants when examined by sodium dodecyl sulphate-polyacrylamide gel electrophoresis⁸. Rabbits were injected with 2 mg of myosin in 0.4 M KCl-0.05 M sodium phosphate buffer, pH 7.4, emulsified with an equal volume of complete Freund adjuvant. After two and four weeks the animals were boosted with the same amount of antigen without adjuvant. Each antiserum, collected in the early stages of immunisation (less than three months), yielded a single precipitin line in agar

Fig. 1 Double immunodiffusion of cardiac and skeletal myosins with anti-ventricular myosin antiserum (a) and anti-fast skeletal myosin antiserum (b). The central wells contained the antisera. The outer wells beginning with the well on the left and proceeding clockwise contained: column-purified ventricular myosin, unpurified ventricular myosin, unpurified atrial myosin, column-purified pectoralis muscle myosin. Immunodiffusion was carried out in 1% agarose in 0.4 M KCl-0.05 M potassium phosphate, pH 7.4. The plates were incubated for 3 d at room temperature.

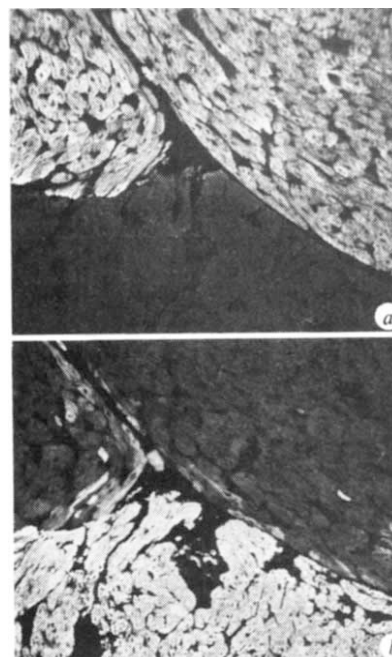
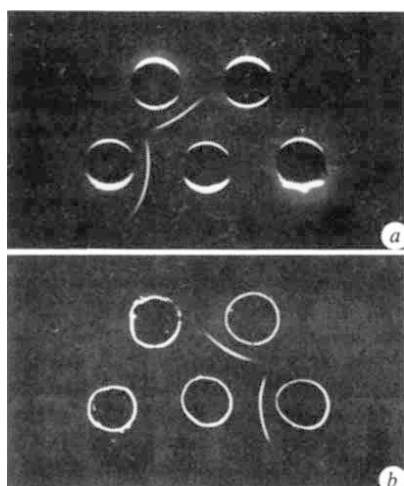


Fig. 2 Direct immunofluorescence assays on serial cryostat sections through a block composed of left atrium and ventricle of the chicken heart. After incubation with fluorescein-labelled anti-fast skeletal myosin antiserum (a), atrial cells (upper field) appear stained whereas ventricular cells (lower field) are unstained. A reversed pattern of staining is seen with fluorescein-labelled anti-ventricular myosin antiserum (b). Sections were incubated for 30 min at room temperature with anti-myosin antisera. After washing, the sections were fixed in 2% paraformaldehyde in phosphate-buffered saline for 10 min, mounted in buffered glycerol and examined using a Leitz fluorescence microscope equipped with incidence fluorescence optics. Magnification $\times 150$.

immunodiffusion and immunoelectrophoresis with the homologous antigen. We have not investigated which portion of the myosin molecule is responsible for its antigenicity using our immunisation schedule, but previous studies^{9,10} suggest that the antibodies are directed against the heavy chains of myosin. Anti-fast skeletal myosin and anti-slow skeletal myosin did not react with ventricular myosin: this indicates, in agreement with previous reports^{10,11}, that chicken ventricles contain a type of myosin antigenically distinct from the myosins present in skeletal muscles. Similar results were obtained using crude myosins, not purified through column, instead of the corresponding purified myosins. However, there was a clear-cut difference between unpurified atrial myosin and ventricular myosin in both immunodiffusion and immunoelectrophoresis reactions. Unpurified atrial myosin reacted with anti-fast skeletal myosin antiserum but not with anti-ventricular or with anti-slow skeletal antisera (Fig. 1).

Fluorescein isothiocyanate was coupled to the IgG fraction from each antiserum and conjugates with an appropriate fluorochrome/protein ratio were obtained as previously described¹². Direct immunofluorescence assays were carried out on fresh-frozen sections of skeletal muscles and heart. Anti-fast and anti-slow skeletal myosin antisera stained selectively fast and slow skeletal muscle fibres, respectively (see also refs 9 and 10). Specificity of immunofluorescence labelling was demonstrated by absence of staining with fluorescent pre-immune serum and by inhibition of the staining reactions by pretreatment of each antiserum with the corresponding myosin. Muscle cells of the ventricles were labelled by anti-ventricular myosin but were not stained by either the anti-slow or the anti-fast skeletal myosin. In contrast, as shown in Fig. 2,

atrial muscle cells did not react with the anti-ventricular nor with the anti-slow skeletal serum, but were brightly labelled by the anti-fast skeletal serum. There was no difference between right and left ventricle nor between right and left atrium in their reactivity with the different antisera. Purkinje cells, which are present in both atria and ventricles of the chicken heart, and can be recognised by their large size and characteristic subendocardial and periarterial distribution^{13,14}, were found to react with all three antisera. They were stained intensely with the anti-slow skeletal serum, moderately with the anti-ventricular serum and variably with the anti-fast skeletal serum (Fig. 3). Reactivity of Purkinje cells with the anti-fast skeletal serum was apparently related to their location within the heart; Purkinje cells in the atria were moderately stained whereas those in the ventricles showed very little reactivity.

To establish whether any single Purkinje cell can react with more than one antiserum, fluorescent tracing of anti-fast and anti-slow skeletal myosin was carried out in sequence in the same section following the method described by White¹⁵. The section was first incubated with anti-fast myosin and labelled Purkinje fibres identified. Ultraviolet illumination was then used to extinguish fluorescence in the chosen field almost completely, then anti-slow skeletal myosin was applied to the section. The same Purkinje cells were now brightly stained. Similar results were obtained with consecutive staining with anti-ventricular and anti-slow skeletal myosin. Single Purkinje cells can thus react with at least two different anti-myosin antisera, presumably as a result of the presence of different myosins in the same cell. The peculiar type of reactivity of

Purkinje cells is a feature they have in common with embryonic cardiac cells¹¹ and may thus be an expression of a primitive organisation of their contractile apparatus. In addition to typical Purkinje cells, there was a minor proportion of muscle cells in the chicken atria which displayed multiple cross-reactivity with anti-skeletal and anti-ventricular myosin antisera. These cells could not be easily distinguished by size and distribution from normal atrial cells and their nature is now being investigated.

The response of atrial muscle cells, indicating marked cross-reactivity of their myosin with the myosin from fast-skeletal muscles, was quite unexpected, as cardiac muscle has generally been associated with slow rather than fast skeletal muscle. Evidence for the presence of a fast-skeletal type of myosin in the atria of the chicken heart was independently obtained from histochemical observations on heart sections incubated for the demonstration of myosin ATPase activity. Histochemical ATPase activity¹⁶ was higher in atrial cells than in ventricular cells, the reaction being unaffected by preincubation with Triton X-100 (ref 17). In contrast with ventricular cells, myosin ATPase activity of atrial cells remained unchanged with pretreatment in alkali (pH 10.5), but was almost completely inhibited with pretreatment in acid (pH 4.35). This is precisely the type of response one would expect in a fast-skeletal muscle (slow muscles showing, in contrast, alkali labile and acid stable myosin ATPase activity¹⁸).

The significance of the difference in myosin composition between atrial and ventricular muscle cells remains to be established. In view of the close association of myosin structure and contractile properties of muscle cells, one would expect the atrial cells to be relatively fast contracting compared with ventricular cells. It is of interest that myosin isolated from the dog heart atria has been found to display different ATPase activity and light-chain pattern from myosin of the ventricles¹⁹. Therefore, cardiac myosin polymorphism apparently represents a feature of mammalian as well as avian heart.

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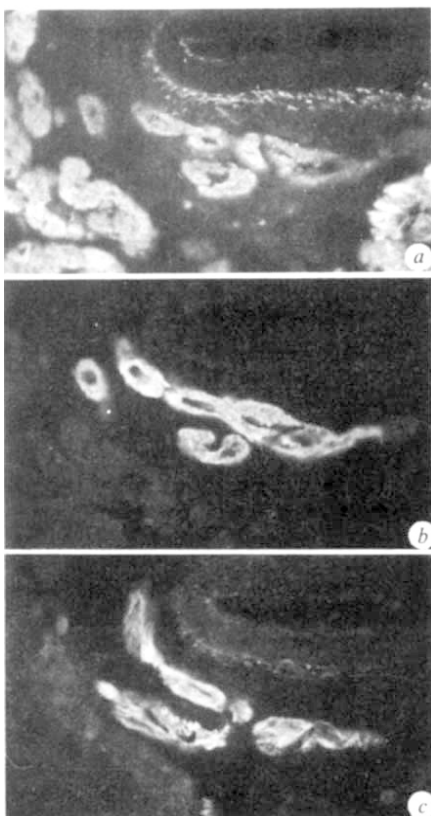
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Fig. 3 Serial non-consecutive sections through a bundle of Purkinje fibres in the left atrium of the chicken heart stained with fluorescein-labelled anti-fast skeletal myosin (a), anti-slow skeletal myosin (b) and anti-ventricular myosin (c). Note staining of surrounding atrial cells only with anti-fast skeletal myosin. Autofluorescence of elastic fibres in the artery in the upper right field. Magnification $\times 300$.



AUG is the only recognisable signal sequence in the 5' non-coding regions of eukaryotic mRNA

THE nucleotide sequence of the 5' non-coding region of messenger RNAs is particularly interesting because it may contain the signals for processing and modification of mRNA, such as its cleavage from precursor and 'capping' (the addition of the 5' terminal m⁷Gppp) and possible recognition and binding sites for ribosomes and initiation factors. We have reported the complete sequence of the 5' non-coding region of rabbit globin mRNA—the first non-viral eukaryotic mRNA¹, followed by similar studies on human and rabbit α and β -globin mRNAs^{2,3}. Here we report the sequence of the 5' non-coding regions of mouse α -globin, β^{min} -globin and β^{maj} -globin mRNA and make a detailed comparative study of this region.

The nucleotide sequences were determined by similar methods to those previously described¹⁻³. ³²P-labelled cDNA deriving specifically from the 5' non-coding regions was prepared by using reverse transcriptase. The template was a mixture of mouse α -, β^{min} - and β^{maj} -globin mRNAs and the primer, the synthetic hexanucleotide d(GCACCA)¹. The synthetic cDNAs, either internally or end ³²P-labelled³, were fractionated on a 12% polyacrylamide gel (see Fig. 1a). Bands 1 and 2 were about the same length and had similar, but not identical, depurination⁴ fingerprints to those previously obtained from human and rabbit globin cDNAs¹⁻³. In this way it was possible to identify bands 1 and 2 as being derived from the 5' non-coding region of β and α -globin mRNA respectively. As the β component was suspected to be a mixture of β^{maj} and β^{min} cDNA a further fractionation of band 1 was carried out by electrophoresis on cellulose acetate⁵. This method is able to discriminate between oligonucleotides of identical length differing in their base compositions (see Fig. 1b). The two bands were assumed to be the 5' non-coding cDNA of β^{min} and β^{maj} -globin mRNA and were arbitrarily labelled A and B because it was not possible to distinguish between them. Quantitation of the radioactivity in bands A and B by direct counting of the bands shows a ratio of 2:3. This might suggest that B is the β^{maj} component. If the β mRNAs are present at the same ratio as their respective cDNAs, a difference in their translational ability must exist to ensure the 4:1 $\beta^{\text{maj}}:\beta^{\text{min}}$ protein ratio found *in vivo* and *in vitro*⁶.

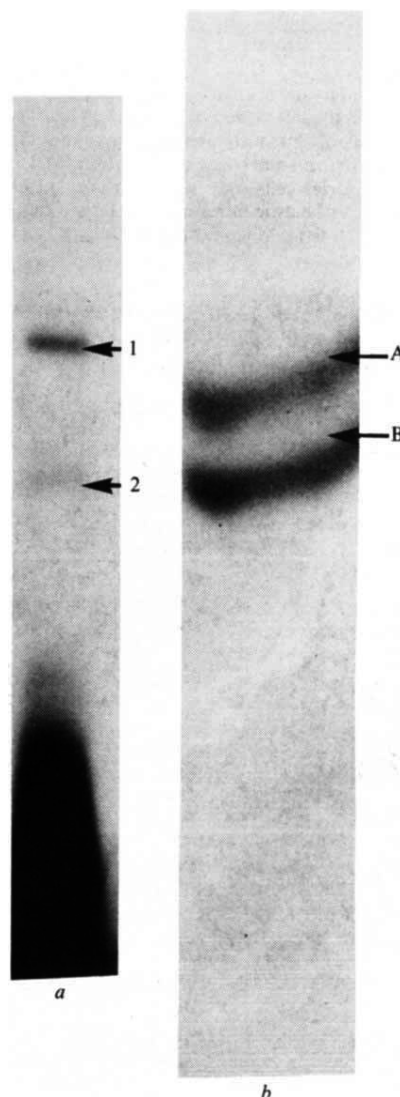
The end-labelled β_A , β_B and α -cDNA bands were subjected to the chemical degradation procedure described by Maxam and Gilbert⁷ and the products analysed in a 12% polyacrylamide gel. Figure 2 (a and b) shows two autoradiographs where almost all the β_A and β_B sequences can be read and Fig. 3 shows the cDNA sequences deduced for all three mRNA molecules, although residue 57 of the β cDNAs remained tentative (see Fig. 2 legend). As a cross check of these sequences, depurination fingerprints of all three cDNAs were analysed and in the case of the α -globin cDNA the sequence was also cross checked by the partially minus method¹⁻³ and partial venom and partial spleen phosphodiesterase digest⁸. The results (not shown) were entirely consistent with the sequences presented in Fig. 3. The sequence m⁷GpppAC was previously determined for the 5' terminal nucleotides of murine α and β -globin mRNA⁹ and this is consistent with the complementary 3' terminal sequence GT deduced here for all three cDNAs.

The 5' non-coding regions of mouse α , β_A and β_B globin mRNAs are presented in Fig. 4. The β_A and β_B sequences differ from one another in eight nucleotides out of 52 (about 85% homologous). Furthermore each mouse β sequence differs extensively from human β mRNA (see Table 1). It was therefore not possible to use sequence homology as a way of

identifying the relationship of the mouse β major and minor system with the human γ , δ and β system.

The genes for β^{maj} and β^{min} are known to be in the same linkage group¹⁰. If they are near each other (that is, in a cluster of genes, perhaps together with the gene coding for the embryonic γ globin chain), they may be served by the same promoter. However, a situation such as that described for adenovirus^{11,12} where a common 5' non-coding sequence is 'spliced' in probably all the late mRNAs, can now be ruled out here because of the considerable differences in the 5' non-coding sequence of β^{maj} and β^{min} -globin mRNAs. It is still possible, though, that other types of DNA rearrangement do take place

Fig. 1a, Autoradiograph of a 12% acrylamide gel fractionation of end ³²P-labelled cDNA. The cDNA either internally or end ³²P-labelled (refs 1-3), was prepared *in vitro* with AMV reverse transcriptase using as template a mixture of mouse α , β^{maj} and β^{min} -globin mRNA (a gift from C. Finnigan and C. Coleclough) and the synthetic primer d(GCACCA). Bands 1 and 2 are the β and α cDNAs; the radioactivity at the bottom of the gel is from unreacted 5' end ³²P-labelled primer. **b**, Autoradiograph of a cellulose acetate electrophoresis fractionation (ref. 5) of β cDNA (a, band 1) in its β^{maj} and β^{min} components, arbitrarily labelled A and B because it was not possible to distinguish between them. The ratio A:B as established by direct counting of the bands is 2:3 (see text).



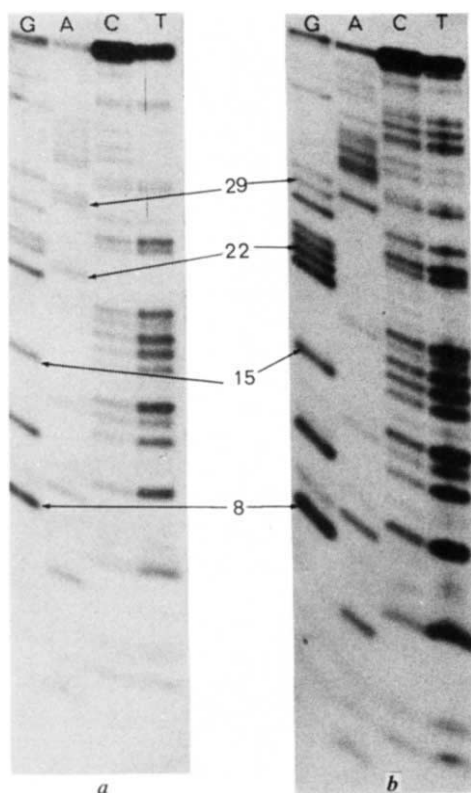


Fig. 2 Gel analysis of Maxam and Gilbert degradations⁷ of β_A (a) and β_B (b) cDNAs. The end labelled cDNA (Fig. 1b, bands A and B) was eluted from the cellulose acetate and the four base-specific degradations⁷ were carried out. G, A, C and T denote G-specific, A>C, C-specific and C+T cleavages. The numbers correspond to the position of that nucleotide in the respective cDNAs (see Fig. 3). The T residues were not suppressed in the C-specific cleavage for reasons which are not understood, but fortunately confirmatory information about C residues can be obtained from the A>C and C+T channels. However, the residue at position 57 in β_A and β_B cDNA remains tentative, although it is most probably a dC in β_A and a dT in β_B .

at the 5' end of the molecule, and the sequence reported here may help to pinpoint them when the cloned gene DNA is sequenced¹³.

The nucleotide sequence of the 5' non-coding region of mouse, human and rabbit globin mRNA is shown in Fig. 4a. We have noted previously that the corresponding regions of rabbit α - and β -globin mRNAs did not present extensive homology². On the other hand, we have shown that the 5'

Table 1 Percentage homology between the 5' non-coding region of globin mRNAs

	β -globin mRNAs				α -globin mRNAs	
	H	M _A	M _B		H	M
R	81.1	71.7	75.5	R	75.7	66.6
H	—	78.9	80.8	H	—	70.3
M _A	78.9	—	84.6			

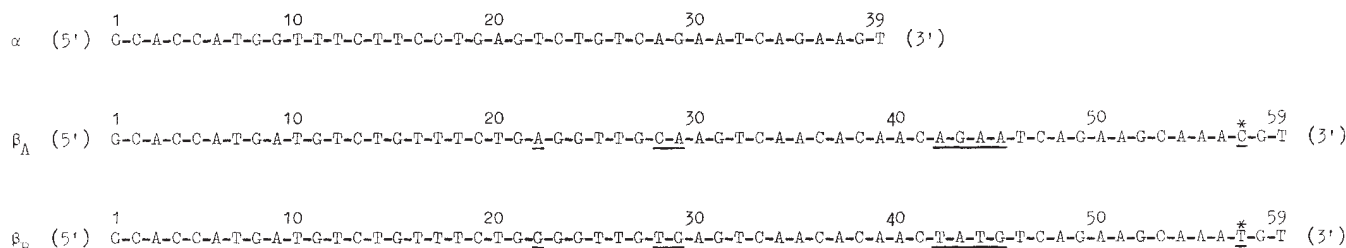
The numbers denote per cent homology and were calculated as follows: The mRNA sequences were aligned (allowing insertions and deletions) to maximise homology. The conserved nucleotides were then counted and expressed as percentage of the total number of positions in the 5' non-coding region. R, H and M denote rabbit, human and mouse.

non-coding region of human and rabbit β -globin mRNAs were about 81% homologous whereas the same regions of human and rabbit α -globin mRNAs were 75% homologous³. The elucidation of the mouse globin mRNA sequences described above now increases the number of comparable examples to seven. This should allow us to assess whether there is any common sequence feature present, which could be related to a common biological function of the messengers.

The most general homology in the 5' non-coding region of all α and β -globin mRNAs analysed is the hexanucleotide CUU-PyUG lying close to the cap structure (solid line boxes in Fig. 4). In addition they all contain a purine-rich region whose 3' end is placed between two and six nucleotides to the left of the AUG (broken line boxes in Fig. 4)—a feature somewhat resembling the situation in prokaryotic mRNA¹⁴. It is interesting to note that this purine-rich region can base pair to form a hairpin loop with the hexanucleotide CUUPyUG and its surrounding sequences. However, the degree of complementarity is variable in the different examples and concerted changes in both regions that will preserve the base pairing are not always observed. The significance of this feature therefore remains an open question. All the β -globin mRNAs shown in Fig. 4a contain regions absent in the α -globin ones. The sequence UGUGUU (underlined in Fig. 4) is particularly interesting because it is conserved in all the species of β -globin mRNA analysed.

The 5' non-coding region sequences of ovalbumin mRNA (L. McReynolds *et al.*, in preparation) and of several viral mRNAs have been elucidated and are shown in Fig. 4b. The VP1 protein mRNA from SV40¹⁵ and TYMV¹⁶ genome RNA show nucleotide sequences surprisingly homologous to the globin mRNAs. Both the sequences CUUPyUG and GUGUU are present. However, ovalbumin mRNA does not possess them and BMV RNA¹⁷, VSV mRNAs¹⁸, reovirus mRNAs^{19,20}, ASV RNA^{21,22}, A1MV RNA²³, TMV RNA²⁴ and TYMV coat mRNA¹⁶ fail to show any obvious homology with the globin mRNAs.

Fig. 3 Sequence of the cDNAs of the 5' non-coding region of mouse α , β_A and β_B globin mRNAs. Numbers denote the distance of the nucleotide at that position from the 5' end. Underlining denotes sequence variation between β_A and β_B cDNAs (see gels in Fig. 2). * Denotes that the identification of the nucleotide at that position is tentative.



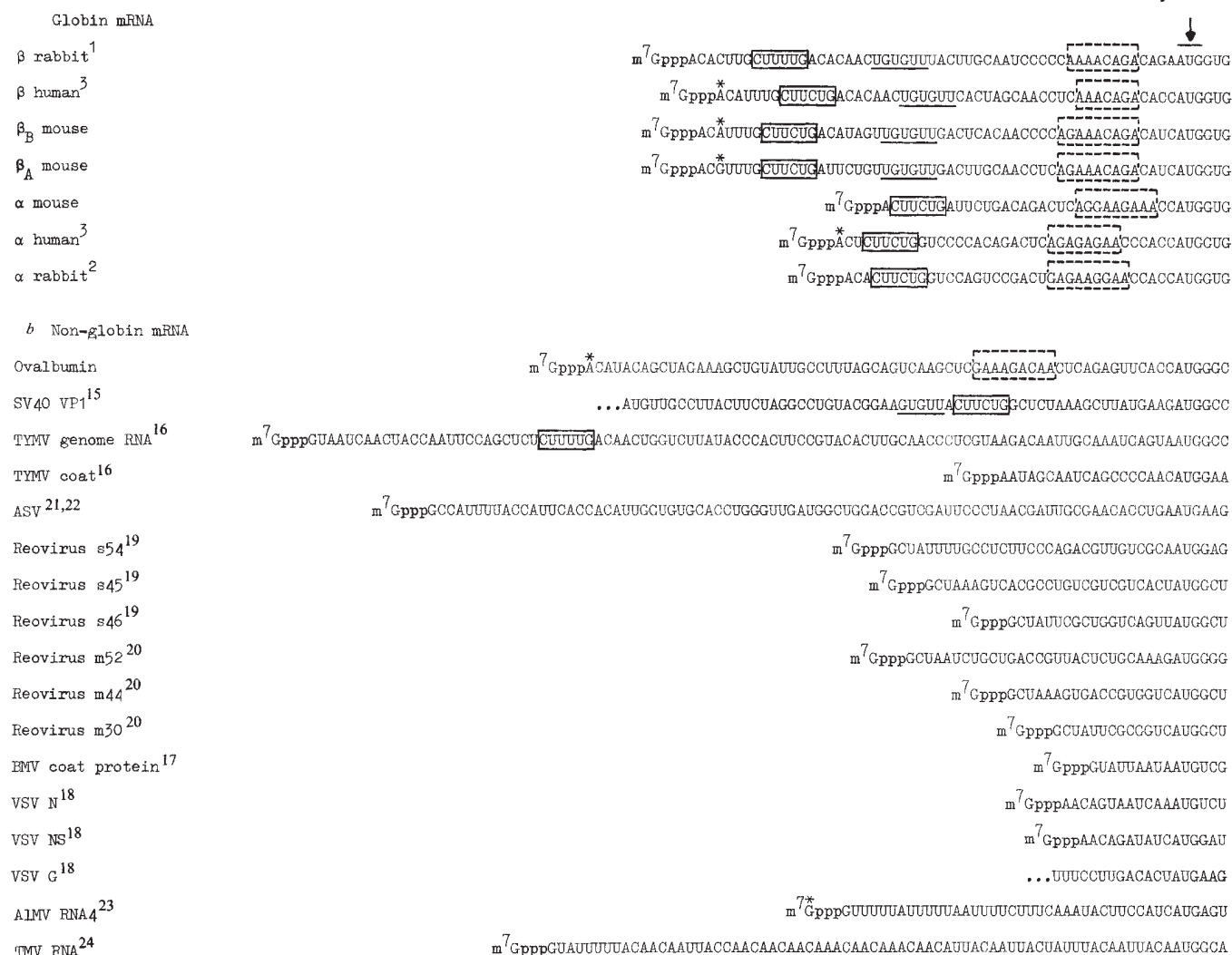


Fig. 4 Nucleotide sequence of the 5' non-coding region of eukaryotic mRNAs. The partial homologies are enclosed in boxes or underlined and are discussed in the text. * Denotes that the identification of the nucleotide at that position is tentative. The arrow points to the initiation codon. The AUG selected as initiation codon in TYMV genome RNA, VP1 and ASV mRNA is the most probable, but in all three cases there are other AUGs on the 5' side^{15,16,21,22}

Shine and Dalgarno²⁵ have suggested that the 5' non-coding region of eukaryotic mRNA may possess a similar type of ribosome binding signal sequence to that present in lower organisms. Because of the difficulty in finding a universally conserved sequence other than AUG (a fact reinforced by the data presented here) we have previously suggested¹⁻³ that the initiator triplet itself may function in a modified Shine and Dalgarno interaction. In this model the AUG of the mRNA first binds to the 3' end of 18S rRNA, which possesses the methionine anticodon sequence 3' UAC 5', thus positioning the mRNA correctly in the small subunit of the ribosome. Subsequently the Met-tRNA_i itself bound to the small subunit, would displace the 18S rRNA to allow translation to proceed.

An alternative model has been proposed as a result of recent sequencing studies at the 3' end of 18S rRNA²⁶. The purine-rich segment 3' GAAGGC 5' present eight nucleotides from the 3' end of 18S rRNA²⁶⁻²⁸ is complementary to the 5' non-coding region of several mRNAs. The best base pairing is observed with the mRNAs that possess the hexanucleotide CUUPyUG. However, not all the mRNAs analysed showed complementarity (for example, BMV, VSV, N and VSV NS) and when they did the 5' end of the complementary regions was at a widely variable distance from the AUG initiating codon (8-44 nucleotides). It seems then unlikely that the observed complementarities are involved in a function strictly identical to the Shine and Dalgarno interaction in bacteria²⁵, where the

5' end of the complementary region is located at a reasonably conserved distance from the AUG initiating codon¹⁴.

Both the models discussed above are speculative because there is no direct evidence as yet that 18S rRNA is involved at all, in the binding of mRNA to the ribosome.

The heterogeneity in the size and in nucleotide sequence of the 5' non-coding regions of mRNAs that use the same ribosomes and the same initiation factors is puzzling. Recent ribosome binding studies using fragments of reovirus mRNAs have shown that the AUG is the only essential feature needed for specific ribosome binding²⁹. However, the efficiency of binding was reduced by removal either of 5' non-coding region sequences, including the cap, or of coding sequences on the 3' side of the AUG. Part of the explanation may be that different mRNAs have quantitatively different requirements for initiation factors³⁰. For example, the sequence CUUPyUG may be specific for an initiation factor in some mRNA species whereas other, perhaps less sequence-specific, signals may be used in other messengers. However, other functions for the 5' non-coding sequences like signals for RNA processing or modification cannot be ruled out. We must also admit that some at least of the sequence might be 'junk'. It may not be necessary to find a precise function for every piece of mRNA sequence. It may just be there as a consequence of the evolution of the coding part of the mRNA, and is carried as a neutral and not disadvantageous sequence in evolution. A somewhat

related evolutionary explanation for the presence of introns in the DNA of eukaryotic genes has been suggested by Gilbert³¹.

It is obvious that comparative studies cannot be exploited further and direct functional studies are necessary to elucidate the meaning of the 5' non-coding sequences.

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A different approach to RNA sequencing

DURING the past few years techniques to simplify the systematic sequence determination of nucleic acids have become available; these involve the use of polyacrylamide gels from which the sequence may be directly and rapidly read off. The most recent of these, applicable to RNA fragments, rely on the base-specific nature of cleavage by T_1 and U_2 RNases^{1,2} and pancreatic RNase and RNase I from *Physarum polycephalum*² by which a spectrum of 5' terminally-labelled fragments may be produced and comigrated with a 'ladder' achieved by incubating the fragment with alkali or hot formamide. We describe here a method for the sequence determination of RNA fragments bearing a blocked 5' terminus, in which the above enzymatic hydrolysis steps and their accompanying problems may be avoided.

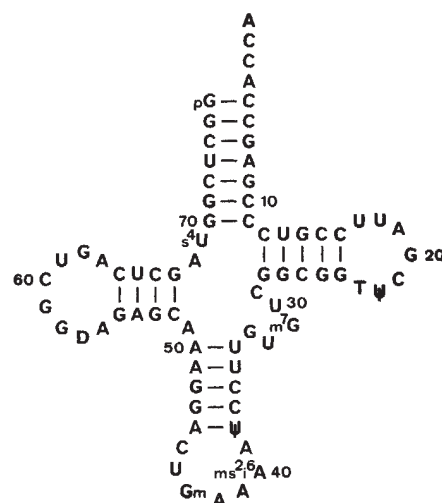
One major problem inherent in the existing techniques is the denaturation of the fragment to ensure that a full complement of partial digestion products may be observed. Work carried out in this laboratory on the 3' terminal fragment of *Bacillus stearothermophilus* 16S rRNA isolated after specific cleavage using colicin E3 (J. S., unpublished) has shown that in cases where strong secondary base-pairing occurs, as has been suggested for this fragment³, enzymatic hydrolysis only occurs significantly at nucleotides found in single-stranded regions, even in the conditions of elevated temperature (50 °C) and in the presence of 7M urea¹. This being the case, gaps appear in the gel pattern where bands are either absent or represented faintly, even though bands are seen in the analogous positions within the 'ladder'. To overcome this problem we have tried a different approach, relying initially not on enzymatic but on chemical methodology, based on the observation that hydrolysis using formamide² gave a complete and reasonably regular spectrum of products even in the presence of strong secondary interactions.

Both sequencing techniques so far cited involve the labelling of the 5' nucleotide of a fragment with subsequent hydrolysis to produce an array of partial products, of which only those containing the 5' label will appear on the autoradiograph of the gel. The technique described in this paper requires the fragment to be hydrolysed before being 5' terminally labelled. If the fragment is treated with formamide a series of subfragments will result, each bearing a 5' hydroxyl group and a 3' phosphate. Theoretically, provided that the degree of hydrolysis is small when compared to the amount of fragment present, the situation will arise in which the number of double 'hits' within any one fragment is insignificant when compared to the number of single 'hits'. Therefore, the relative amounts of products originating from within the fragment will be small when compared to those encompassing either the 5' or 3' termini and furthermore, assuming the presence of a 5' phosphate on the original fragment, only those products from the 3' terminus will have a 5' hydroxyl group. The outcome of T_4 polynucleotide phosphokinase treatment of the formamide hydrolysate in the presence of [γ -³²P]ATP may be expected to be a series of ³²P-labelled oligonucleotides each containing an identical 3' terminus, differing in length only at the 5' (labelled) end. Fractionation of the mixture on a denaturing polyacrylamide gel should give the familiar ladder, and the 5' nucleotide of each band within the ladder may be determined by elution of the material and subsequent analysis after complete alkaline hydrolysis. Thus the sequence of the fragment may be read in the direction 3' to 5' with respect to increasing product size.

To investigate the feasibility of the technique, purified tRNA^{phe} from *B. stearothermophilus* was used; the sequence of this molecule (Fig. 1) has been determined by conventional techniques⁴.

Amounts of RNA varying from 0.1 µg to 10 µg were dried under vacuum and treated with hot formamide. When sufficient quantities of material were used, the formamide was removed by ethanol precipitation of the RNA—a rapid procedure but with the disadvantage of a low recovery of the mono-, di- and trinucleotides. The alternative approach is to lyophilise the sample, a volume of 10 µl requiring about a day for completion. This however has the advantages that small amounts of RNA may be used that could not be precipitated using ethanol and that small products of hydrolysis are not lost. Preliminary experiments have shown that a 10% (v/v) solution of formamide does not noticeably reduce the efficiency of the subsequent T_4 polynucleotide phosphokinase reaction, and

Fig. 1 The primary nucleotide sequence of tRNA^{phe} from *B. stearothermophilus* arranged in the cloverleaf configuration. The numbering system on this and subsequent figures is from 3' to 5' to facilitate description of the method, the smallest fragments, and hence those initially investigated, originating from the 3' terminus.

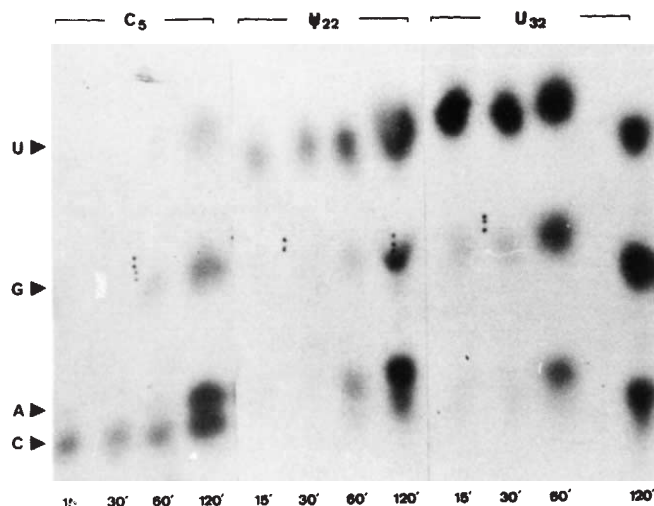


consequently its complete removal is not absolutely necessary.

To find the optimum conditions for formamide hydrolysis, the RNA was treated at 100 °C for various times, and after gel fractionation analogous bands from adjacent slots were analysed for the 5' terminally labelled nucleotide, examples of which are shown in Fig. 2. As predicted from the theoretical considerations, with increasing times of hydrolysis, the amount of nonspecific background contamination increases to the point where it appears in yields similar to, or at times in excess of, the expected nucleotide. Conditions for routine hydrolysis were chosen to give reasonably clean spots on analysis, whilst allowing the production of sufficient amounts of the partial products for subsequent T₄ polynucleotide phosphokinase labelling and analysis.

The fractionation pattern obtained after formamide treatment at 100 °C for 15 min is shown in Fig. 3 and the analysis of the labelled nucleotides after elution from the gel and subsequent alkaline hydrolysis, in Fig. 4. The four common nucleotide diphosphates are sufficiently well fractionated by this method to enable the sequence to be read off directly. With two exceptions, the sequence of nucleotides 2 to 75 may be deduced from the data given in Fig. 4, the correct 5' nucleotide in each case being the product that appears in the highest yield. The two ambiguous results concern fragments 38 and 69, the expected 5' terminal nucleotides being pUp and ps⁴Up (or its degradation product in the conditions of isolation; pUp) respectively. Analysis of fragment 38 gives the additional products pAp and pGp and fragment 69, pGp. The reasons for the high levels of these products are not clear. In both cases however, the correct 5' terminal nucleotide may be deduced after digestion of the fragment with either T₁ or pancreatic RNase and subsequent electrophoretic fractionation on DEAE-cellulose paper in 7% formic acid. With a knowledge

Fig. 2 The effect of increasing times of formamide hydrolysis on fragment specificity. Aliquots of 10 µl RNA were incubated at 100 °C for 15, 30, 60 and 120 min in 10 µl deionised formamide⁵. The hydrolysate was precipitated with 2.5 volumes ethanol after the addition of 0.05 volume 3M NaCl and the precipitate was washed once with ethanol and dried. The material was dissolved in 10 µl twice concentrated buffer (20 mM Tris-HCl pH 8.0, 20 mM MgCl₂, 12 mM mercaptoethanol) to which was added 3 µl T₄ polynucleotide phosphokinase (1 unit µl⁻¹) and 7 µl [γ-³²P]ATP (2nM, 400 Ci mmol⁻¹). The mixture was incubated at 37 °C for 30 min and 4 µl aliquots (2 µg RNA) were mixed with 6 µl formamide-dye mix⁵ and loaded onto a 20% polyacrylamide gel containing 7M urea (gel dimensions: 40 × 24 × 0.2 cm). The gel was run at 600 V for 20 h. Adjacent bands were selected from throughout the gel, the RNA eluted, hydrolysed using alkali and the resultant ³²P-labelled nucleotide diphosphates fractionated by electrophoresis on 3MM paper at pH 3.5.



of the sequences adjacent to the nucleotide in question, the correct enzymatic digestion product may be found directly from its mobility relative to the digestion products of the neighbouring fragments.

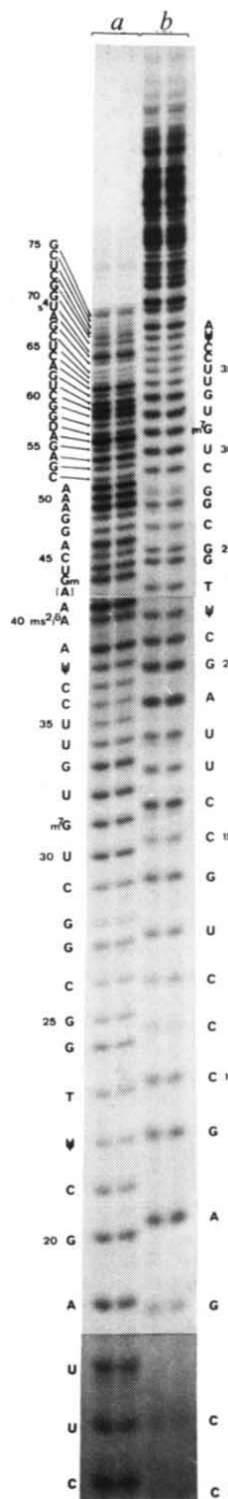
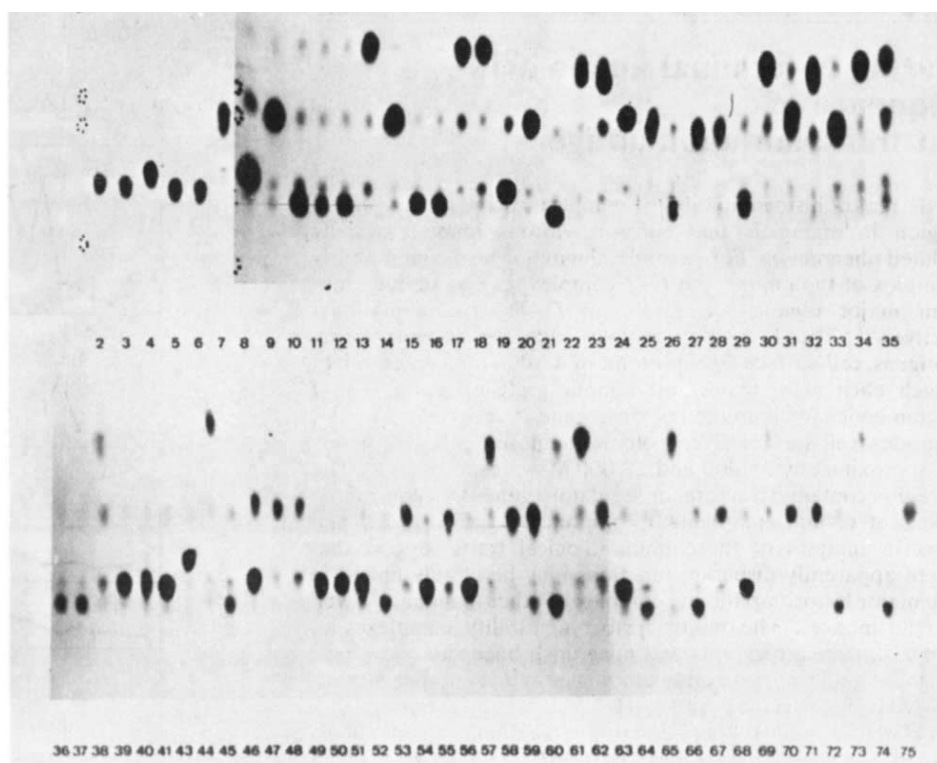


Fig. 3 The fractionation pattern obtained after incubation of 10 µg tRNA^{phe} at 100 °C for 15 min in 10 µl deionised formamide. The labelling procedure was as for Fig. 2. Two identical aliquots (a) were loaded onto a 20% polyacrylamide gel containing 7M urea (gel dimensions: 90 × 24 × 0.2 cm) and migrated for 24h at 1,000 V before loading two more aliquots (b). Electrophoresis was carried out for a further 48h at 1,500 V. To obtain the autoradiograph shown, films were exposed to the gel for 90 min.

Fig. 4 Analysis of the 5' terminal nucleotides of the fragments fractionated as in Fig. 3. The bands were excised from the gel, homogenised with a glass rod and the material eluted with 1 ml 300 mM NaCl, 0.1% sodium dodecyl sulphate by shaking overnight. Gel pieces were removed by passing the solution through a pasteur pipette containing glass wool, 50 µg carrier tRNA was added and the RNA precipitated with 2.5 volumes ethanol. The precipitate was dissolved in 10 µl 0.3M NaOH and hydrolysed overnight at 37°C. The resultant ³²P-labelled nucleotide diphosphates were fractionated by electrophoresis on 3MM paper using the standard pH 3.5 buffer system with EDTA added to a final concentration of 1 mM.



Modified nucleotides create an additional problem concerning product fractionation because of anomalous migration behaviour in these conditions in addition to their frequent instability during isolation. Of these modified nucleotides, pψp_{22,38} and pTp₂₃ migrate slightly slower than pUp and pms²¹iAp₄₀ just ahead of pAp. m⁷G₃₁ is degraded in alkaline conditions to give the product 4-amino-5(*N*-methyl)formamidoisocytosine ribotide⁶, the diphosphate showing a mobility slightly greater than pGp. The diphosphate form of the alkaline derivative of D₅₇, β-ureidopropionic acid *N*-ribotide⁷ migrates to approximately the same position as pUp.

In general, the formamide treatment gives rise to a good spectrum of partial products as determined visually from the mobility shifts on addition/removal of single nucleotides to/from the 5' terminus. Variations of band intensity originate from the nonuniform efficiency of T₄ polynucleotide phosphokinase labelling of the fragments, the degree of labelling being strongly dependent on the nature of the 5' nucleotide. In particular, 5' terminal adenosine residues are readily phosphorylated and invariably appear as relatively intense bands on the autoradiograph. Unless correct conditions are used for the initial formamide treatment the presence of non-specific fragments bearing a 5' adenosine residue can effectively mask the correct nucleotide. Two noticeable anomalies are apparent within the band spacing pattern of Fig. 3, between bands 39 and 40 and bands 41 and 43. Nucleotide 42, an adenosine residue, is not produced as a 5' terminal nucleotide because of the resistance of the phosphate linkage between it and the adjacent nucleotide 2'-*O*-methylguanosine (Gm). Analysis of the 5' nucleotide of band 43 gives a spot migrating slightly faster than pAp, corresponding to the dinucleotide pGmpAp. The unusually large jump between bands 39 and 40 is accounted for by the loss of the hyper-modified nucleotide pms²¹iAp from the 5' terminus.

Smaller labelled hydrolysis products are sometimes obscured by unreacted [γ-³²P]ATP using the gel fractionation system. The data given in Fig. 4 for nucleotides 2 to 7 are derived from a two-dimensional procedure using electrophoresis on cellulose acetate strips at pH 3.5 in the first dimension and homochromatography on DEAE-cellulose thin layer plates in the

second dimension⁸. Further information will be published elsewhere in conjunction with a description of additional applications of the general technique.

The major limitation of this technique is the degree of fractionation of consecutive large fragments by polyacrylamide gel electrophoresis making band excision increasingly more difficult. Although background contamination may occasionally create difficulties of interpretation (in this example, the possible misreading of 2 nucleotides in 74) the technique appears ideally suited to the analysis of small RNA molecules that may be isolated directly with a 5' terminal phosphate group (for example tRNA and 5S rRNA). Furthermore, this approach may be applied as a complementary technique to the established gel sequencing methods, supplying sequence data concerning the 3' termini of large RNA fragments which would otherwise be outside the limit of gel resolution.

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Partial N-terminal amino acid sequence of rat transplantation antigens

THE major histocompatibility complex (MHC) is a genetic region in mammals that controls various immunologically related phenomena. For example, the major histocompatibility complex of the mouse, the *H-2* complex, can be divided into four major regions—*K*, *I*, *S* and *D*—by recombinational analysis^{1,2}. The *K* and *D* regions code for transplantation antigens, cell-surface glycoproteins of 45,000 molecular weight which elicit skin, tissue and tumour graft rejection. The *I* region codes for immune response genes and for the Ia polypeptides, cell-surface glycoproteins composed of heterodimers of approximately 35,000 and 28,000 MW, respectively^{3,4}. The *S* region contains structural or regulatory genes that control the levels of certain complement components^{5,6}. Functional and genetic analyses of these immunological traits suggest that these apparently disparate functions may be closely linked in the major histocompatibility complex of other mammals as well as the mouse⁷. The major histocompatibility complexes of humans, guinea pigs, rats and mice have been shown to have certain significant structural homologies to one another. In rats, the MHC is designated AgB or H-1 (refs 8 and 9, respectively). To date, at least 13 haplotypes, or combinations of allelic, linked genes within AgB, have been identified, based on data from skin grafting and serological testing (ref. 10 and O. Stark, personal communication). Here we present the partial N-terminal sequences of the transplantation antigens isolated from rats of the AgB4 haplotype. Our results demonstrate that the rat AgB4 transplantation antigen is homologous to those of the mouse, human and guinea pig. The sequence data also suggest that at least two major components are present. These sequences may represent the rat homologues of the mouse *K* and *D* transplantation antigens.

The transplantation antigens coded by the AgB complex are of particular interest because they seem to differ from their mouse counterparts in one fundamental property, namely, the extent of genetic polymorphism which they exhibit. In inbred mice there are at least 11 *K* alleles and 10 *D* alleles which form 25 or more haplotypes. Immunogenetic and biochemical analyses of the mouse transplantation antigens show that there

Fig. 1 SDS-PAGE profile of BN.B4 molecules precipitated by (Lewis × BN.B2) F1 anti-DA antiserum. Triton X-100-solubilised antigens from 7.5×10^5 ³H-tyrosine labelled BN.B4 spleen cells were incubated with 20 μ l antiserum overnight at 4°C. Antigen-antibody complexes were collected by adsorption to *S. aureus* Cowan I¹⁸, and separated on a 10% gel in the presence of 2-mercaptoethanol. The AgB4 transplantation antigen and Ia-like antigens are shown. Immunoglobulin heavy chain, light chain, and ovalbumin markers are shown by arrows.

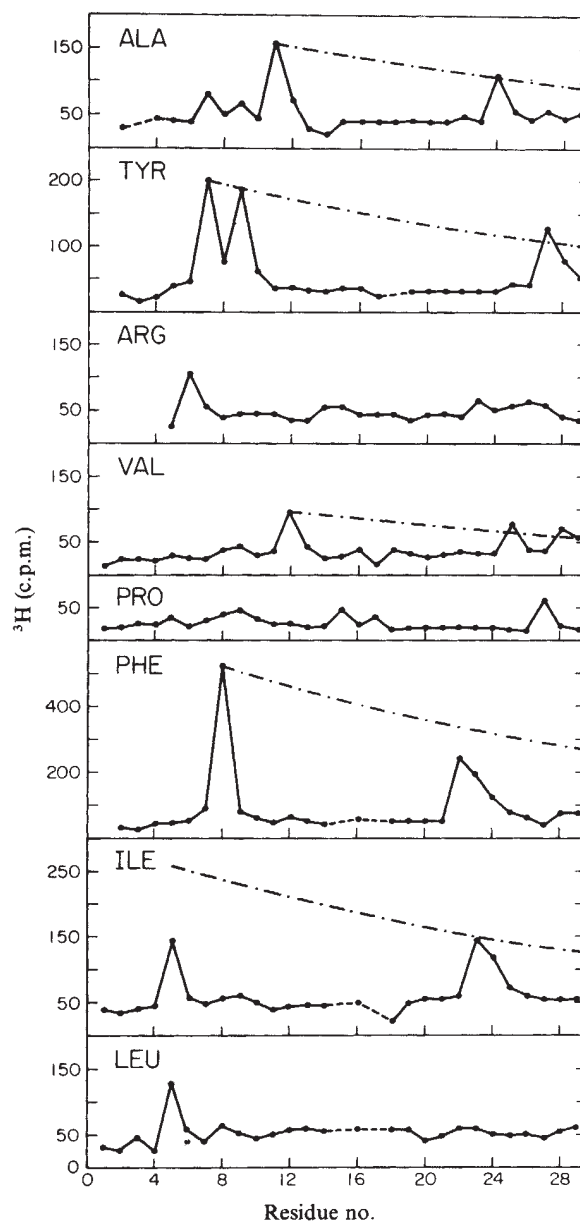
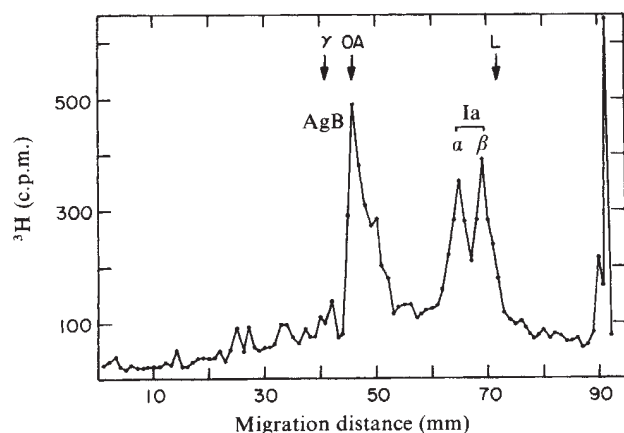


Fig. 2 Microsequence analysis of AgB4. The amount of radioactivity associated with each of the seven incorporated amino acids is plotted against residue number. Repetitive yields (---) are calculated where two or more residues are identified. The isoleucine profile shows residues of roughly equal yields at positions 5 and 23. The simplest explanation for this anomaly is that there are two major sequences being analysed and both sequences have isoleucine at position 23, whereas only a single sequence has isoleucine at position 5. Hence, the repetitive yield curve is drawn from the residue at 23, presumably shared by both major sequences. The chromatography gradient failed to separate adjacent residues at several positions (alanine at position 2, tyrosine at 18, and phenylalanine, isoleucine and leucine at positions 15 and 17). These uncertainties are indicated by broken lines and no residue assignments are made at these positions. The minor peaks for alanine at positions 9 and 11 arise as spillover from the adjacent tyrosine peaks in the chromatography separation. Arginine co-chromatographed with phenylalanine in positions 1–4, and subsequently it was resolved from other labelled amino acids. There is no radioactive peak in the phenylalanine profile over steps 1–4. The phenylalanine at position 23 drops slowly over the next few steps due to the 'lag' that has accumulated over 23 residues because at each step 1–2% of the N-terminal amino acid is not cleaved and this causes an accumulating percentage of the polypeptide to be out of phase.

AgB4 antigens were isolated from biosynthetically labelled BN.B4 spleen cells by immune precipitation. The BN.B4 strain has been made partially congenic for the AgB4 haplotype by backcrossing (BN $\times$ DA)F1 to BN for 11 generations before breeding *inter se* to obtain homozygotes. The antiserum was prepared by skin grafting followed by immunisation of (Lewis $\times$ BN.B2)F1 rats with DA (AgB4) lymphocytes. The profile of the BN.B4 immune precipitate separated by polyacrylamide gel electrophoresis in sodium dodecyl sulphate (SDS-PAGE) is shown in Fig. 1. In addition to the 45,000 MW component, the presumptive transplantation antigens, this antiserum detects Ia-like molecules of 28,000 and 35,000 MW. The β -2-microglobulin component of 12,000 MW associated with the 45,000 MW component runs at the dye front on 10% gels. Its presence has been confirmed on 15% gels. The (Lewis $\times$ BN.B2)F1 anti-DA antiserum has potential reactivity with private specificity 4 and with the public specificity 30⁸ when tested on BN.B4 spleen cell lysates. We are confident that this antiserum detects the AgB transplantation antigen for two reasons. (1) The antiserum was prepared in and tested on cells from animals essentially congenic except for the AgB complex, thus virtually eliminating the detection of antigens coded by genes that are not closely linked to AgB. (2) The same 45,000 MW component is completely cleared from the soluble antigen preparation by precipitation with an antiserum

The partial amino acid sequence data from rat transplantation antigens are presented with data from their mouse, human and guinea pig counterparts in Fig. 3. Three points are of interest. First, the rat AgB4 sequences are clearly homologous to the MHC transplantation antigens of other mammals. For the rather small number of residues identified, the rat transplantation antigens show between 60% and 80% sequence identity with other mammalian transplantation antigens. Indeed, each residue present in the rat molecules is present in one of the other mammalian transplantation antigens. These homologies demonstrate clearly that the genes encoding the transplantation antigens from mouse, human, guinea pig and rat descended from a common ancestor. Second, the presence of leucine and isoleucine at position 5 suggests that we have isolated at least two transplantation antigens which are identical at 12 of 13 positions compared. The leucine-isoleucine polymorphism has been noted in the sequence of transplantation antigens from rats of the AgB5 haplotype (data not shown) and in the transplantation antigens from the guinea pig^{14,15} (Fig. 3). Accordingly, this

Position

[illegible]

polymorphism seems to be present in the transplantation antigens of several rodents. It is likely that these sequences represent two major transplantation antigens, perhaps equivalent to the *K* and *D* antigens of the mouse. Finally, it is interesting to note that there is only one residue difference in 12 (~8%) noted between the two rat transplantation antigens. After more complete sequence analysis it will be interesting to determine whether the rat transplantation antigens differ as extensively as their mouse counterparts⁴.

Obviously, more data will be required before firm conclusions can be reached on the sequence diversity of individual rat transplantation antigens and on the implications this diversity has for the extent of polymorphism this system exhibits. The use of monoclonal antibodies to transplantation antigens may allow us to separate individual gene products¹⁶. In addition, we hope to make use of recombinants in the AgB complex to determine whether the genes encoding the transplantation antigens are both on the same side of the existent recombinants¹⁷. This will be possible if the putative *K* and *D* homologues in the rat exhibit haplotype-associated sequence differences. More detailed amino acid sequence and comparative peptide map studies are in progress.

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Evolutionary substitutions and the antigenic structure of globular proteins

A FEW years ago, Crumpton¹ and Reichlin² reviewed information about the antigenic structure of globular proteins of known amino acid sequence and three-dimensional structure. It seemed that only a small fraction of the amino acid residues in such proteins participated directly in antibody binding. This impression was reinforced by Atassi and co-workers, who proposed antigenic structures of two such proteins—sperm whale myoglobin³ and chicken lysozyme *c* (ref.

4). These workers claim to have identified all those amino acid residues which bind to antibodies present in antisera directed against the protein. These claims stem from extensive studies with chemically modified forms of the protein as well as with antigenically active fragments obtained by specific cleavage of the protein. The antigenic residues are arranged in groups called determinants, each of which binds a specific class of antibodies. A typical determinant contains four to seven residues. For both lysozyme and myoglobin, the proportion of antigenic residues is about 15% of the total number of amino acid residues in the protein^{3,4}. Information has also accumulated concerning the power of antisera to detect evolutionary substitutions of amino acids in globular proteins. Quantitative immunological comparisons of related proteins of known amino acid sequence have consistently shown a strong correlation between degree of sequence difference and degree of antigenic difference. This has been demonstrated most thoroughly for monomeric globular proteins such as lysozymes^{5,6}, ribonucleases⁷, azurins⁸ and cytochromes *c* (refs 9, 10). Less complete information suggests that the correlation holds also for plastocyanins¹⁰, tryptophan synthetase α subunits¹¹, serum albumins¹², carbonic anhydrases and myoglobins¹³ (A. B. Champion, E. M. Prager, S. L. Welch, and A. C. Wilson, unpublished), and ferredoxins¹⁴. From the strength of the correlations observed for lysozymes, ribonucleases, azurins and cytochromes ($r > 0.9$), one may make the statistical inference that about 80% (that is, 0.9 squared) of those amino acid substitutions which accumulate during evolution are immunologically detectable⁷. Here we summarise additional evidence that the majority of evolutionary substitutions are immunologically detectable, and we discuss how to reconcile this evidence with the chemical evidence that only a small fraction of the amino acids are antigenic.

First, we consider the results that Hurrell *et al.*¹⁵ obtained from immunological comparisons of distantly related myoglobins. These workers found that complete sequence homology within the determinant regions defined by Atassi³ does not guarantee immunological cross-reactivity between two myoglobins. To illustrate, antiserum to sperm whale myoglobin does not react at all by radio-immunoassay procedures with cattle myoglobin. Thus the myoglobins of these two species behave as if they had no determinants in common. Yet these two myoglobins are identical in the sequence of amino acids in one of the antigenic determinants delineated by Atassi³. It seems that substitutions elsewhere in the protein have altered markedly the antigenic activity of this determinant.

We next consider closely related pairs of proteins. Turkey and chicken lysozyme differ by seven amino acid substitutions¹⁶ and can easily be distinguished from one another immunologically⁹. Yet none of the seven substitutions occur in the antigenic determinants of chicken lysozyme identified by Atassi and Habeeb⁴. Since turkey and chicken lysozyme are distinguishable antigenically, substitutions outside the determinants may exert some effect on the properties of the determinants. Similarly, bobwhite quail¹⁷ and California quail⁶ lysozymes differ from chicken lysozyme both antigenically and in amino acid sequence, but they are identical to chicken lysozyme at all 17 of its putative antigenic positions.

The problem of reconciling the evolutionary evidence with the chemical evidence is further illustrated by reviewing the capacity of immunological techniques to detect antigenic differences among additional pairs of closely related proteins. Table 1 lists all the known cases in which pairs of closely related, naturally occurring, globular monomeric proteins have been compared by the micro-complement fixation test. This test is highly sensitive to small sequence differences between proteins^{18,19}. By closely related proteins we mean those which are more than 90% identical in amino acid sequence. The list includes 11 pairs of azurins, lysozymes and ribonucleases^{5,7,8}. All of these pairs are distinguishable antigenically.

To approach the problem of calculating the combined probability that all the protein pairs listed in Table 1 would differ

Table 1 Antigenically non-identical pairs of closely related monomeric proteins of known amino acid sequence

Proteins compared*	No. of amino acid differences	Probability of antigenic non-identity (1 - P ₀)†‡	
	(x)	n/N = 0.15	n/N = 0.80
Lysozymes			
1. California quail vs. bobwhite quail	2	0.28	0.96
2. Bobwhite quail vs. chicken	4	0.48	1.00
3. Chicken vs. California quail	4	0.48	1.00
4. Chicken vs. Japanese quail	6	0.63	1.00
5. Duck II vs. duck III	6	0.63	1.00
6. Chicken vs. turkey	7	0.69	1.00
7. Guinea fowl vs. bobwhite quail	8	0.74	1.00
Ribonucleases			
8. Camel vs. dromedary	1	0.15	0.80
9. Goat vs. gnu	3	0.39	0.99
10. Cow vs. goat	4	0.48	1.00
Azurins			
11. <i>Pseudomonas fluorescens</i> C18 vs <i>P. fluorescens</i> C191	4	0.48	1.00
Combinations of independent pairs			
Lysozyme (pairs 1, 4 and 5)	—	0.11	0.96
Ribonuclease (pairs 8 and 9)	—	0.06	0.79
All proteins (pairs 1, 4, 5, 8, 9 and 11)	—	0.003	0.76

* Length of polypeptide chains as follows: lysozyme, 129 residues; ribonuclease, 124 residues; azurin, 128 residues.

† For each of the 11 individual pairs of proteins, equation 3 was used to calculate P₀. For the combinations of independent pairs, equation 4 was used. In every case, it was assumed that the proteins, regardless of their length, contained either 15 antigenic residues per 100 residues of sequence, that is, N = 100, n = 15, or 80 antigenic residues per 100 residues of sequence, that is, N = 100, n = 80.

‡ In order to use these equations, it was necessary to assume that all positions in a protein are equally prone to evolutionary substitution. To check whether this oversimplification is justifiable, we also calculated 1 - P₀ values for the case where only 60% of the positions are assumed to be variable (for N = 60); in this case the 1 - P₀ values are slightly higher but P' for all independent pairs remains less than 0.03 when n = 15. We also asked whether there could be a bias towards occurrence of evolutionary substitutions at those positions which have been shown experimentally to be antigenic in lysozyme. For the lysozymes compared in Table 1, we detected no significant bias in this direction. So, although our use of equations 1-4 entails simplifying assumptions, they seem justifiable in this case.

antigenically, we reasoned as follows. Consider a globular protein N residues in length. Let n be the number of amino acid positions that are antigenic. It follows that N - n is the number of positions which are not part of antigenic determinants. If two related proteins differ by x number of amino acid substitutions, the probability that y of these substitutions occur in antigenic determinants is given by a hypergeometric distribution²⁰:

$$P_y = \frac{\binom{n}{y} \binom{N-n}{x-y}}{\binom{N}{x}} \quad (1)$$

When N is large (>25) the hypergeometric distribution is estimated efficiently by the binomial distribution:

$$P_y = \binom{x}{y} \left(\frac{n}{N}\right)^y \left(1 - \frac{n}{N}\right)^{x-y} \quad (2)$$

For the probability that none of the amino acid replacements fall within an antigenic determinant, that is, y = 0, equation (2) reduces to:

$$P_0 = \left(1 - \frac{n}{N}\right)^x \quad (3)$$

Equation (3) gives the probability that two proteins which differ by x amino acid substitutions will be antigenically identical, that is, that the substitutions occur only outside antigenic determinants.

Table 1 gives for each pair of closely related proteins the observed number of substitutions (x) and the calculated probability of antigenic non-identity (1 - P₀). In general, the probabilities are high when n is 80 for all values of x and relatively low when n is 15 (especially when x is 1 or 2).

The bottom three rows of Table 1 give the joint probabilities of immunological non-identity. These are calculated with equation (4), which gives the product of selected 1 - P₀ values:

$$P' = \prod_{i=1}^j (1 - P_{0i}) \quad (4)$$

P' is the joint probability that j independent pairs of proteins will each have at least one amino acid substitution in an antigenic position and consequently that every pair will be antigenically non-identical. The independent pairs subjected to the combined probability calculation are shown by number in the lower part of Table 1. When n is 80, the combined probability that all 6 independent pairs would be immunologically non-identical is high (P' = 0.76). Thus, the expectation based on the assumption that n is 80 is consistent with the observation that all these closely related pairs of proteins are indeed distinct antigenically. In contrast, when n is assumed to be 15, the probability that these proteins will be antigenically distinct is extremely low (P' = 0.003).

The results of this quantitative test reinforce the impression that the majority of evolutionary substitutions in globular proteins are antigenically detectable. Yet chemical evidence indicates that a minority of the amino acid residues in such proteins participate directly in the antigen-antibody reaction¹⁻⁴. How can these two apparently incongruous lines of evidence be reconciled?

It is possible that most evolutionary substitutions produce subtle, long-range conformational changes in globular proteins. Thus, substitutions occurring outside antigenic determinants may bring about conformational changes in those determinants. With this hypothesis one can explain the power of antisera to detect the majority of evolutionary substitutions while preserving the idea that there are very few antigenic determinants on typical globular monomeric proteins. Although the antigenic effects of substitutions outside determinants could be due in theory to mechanisms not involving conformational change, some immunochemists will find the conformational hypothesis attractive because it is well known that the reactivity of antigenic determinants on globular proteins and many synthetic polypeptides usually depends strongly on their conformation^{21,22}. In fact, Atassi has proposed that the antigenic differences among related proteins from different species are due in large part to conformational differences²³.

There is a difficulty with the conformational hypothesis, however. There is no firm evidence to indicate that the majority of evolutionary substitutions produce significant changes in the conformation of the polypeptide backbone of globular proteins. Very high resolution NMR and X-ray studies of closely related pairs will be required to test adequately the conformational explanation for the ability of immunological methods to detect most evolutionary substitutions in monomeric globular proteins.

An alternative hypothesis must be considered as well. This will be especially necessary if no conformational differences are demonstrable between protein homologues which differ solely by substitutions outside antigenic determinants, and if the magnitude of the antigenic effects of such substitutions prove consistently to be as large as those within known determinants⁶.

The multideterminant hypothesis^{7,8,24} is of particular interest. According to this hypothesis, immunochemists have not yet delineated completely the antigenic structure of proteins such as lysozyme. Besides the determinants identified thus far, there

may be many other determinants, eliciting the production of small amounts of antibody active in micro-complement fixation and embracing collectively almost the entire surface of the protein antigen. Although a thorough search for minor determinants has not yet been carried out, we point out that there is already evidence for the existence of minor determinants associated with the loop region^{22,25} and the active site^{26,27} of lysozyme.

We have used both a qualitative approach and a quantitative one, based on the hypergeometric distribution, to test the hypothesis that a minority of the residues in globular proteins are antigenic. The results indicate that this hypothesis does not by itself account for the capacity of antisera to detect evolutionary substitutions in lysozyme, ribonuclease and azurin. When additional data on the immunological cross-reactivity of monomeric proteins differing by a very small number of amino acid substitutions become available, a stronger test will be possible.

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Immunofluorescence in osteoarthritis

OSTEOARTHRITIS is widely regarded as a degenerative disorder¹, but histological examination shows an inflammatory reaction in the synovium and subarticular marrow in many affected joints. In studies seeking to distinguish between inflammatory and degenerative cases, we have found and

report here the presence of antibody in plasma cells in the synovium in osteoarthritis.

At the stage when osteoarthritic hip joints are treated by prosthetic replacement, most synovia contain a cellular infiltrate of lymphocytes and plasma cells which ranges from light to very heavy, when their appearances become indistinguishable from those of rheumatoid arthritis. We expected the plasma cells to contain immunoglobulins and tested this hypothesis by examining 121 synovia resected during prosthetic replacement of the hip. Tissues were put in cold Ringers' solution in the operating theatre. Two samples of a few millimetres square were selected in the laboratory; one was for formalin fixation and paraffin sections and the other was placed in TC 199 and snap frozen in liquid nitrogen for frozen sections.

The paraffin sections showed that 108 of the synovia were infiltrated by a few to numerous lymphocytes. Plasma cells were present in most and were abundant in 30. Fluorescence of plasma cells was seen in 28 synovia when frozen sections were treated with fluorescein-labelled polyvalent antiserum (Wellcome antihuman immunoglobulin (sheep)). These synovia were then treated with antisera against IgG, IgM, fibrin and complement (Wellcome anti-human IgG (sheep), anti-fibrin, anti-IgM and anti-C₃, fluorescein-labelled). All 28 were positive for IgG and 24 for IgM. Extracellular deposits of complement and fibrin were found in 12 and 27, respectively. Known rheumatoid arthritis synovia were used as positive controls throughout.

These findings of antibody reactions involving complement and with a pattern similar to rheumatoid factor necessitated a careful study for unsuspected rheumatoid arthritis. The clinical notes established beyond doubt that 108 of the cases were definite osteoarthritis. The synovia of 24 of these contained immunoglobulins. As a means of resolving the doubt about the classification of the remaining 13 cases, all the hip joint X rays were divided independently into post-inflammatory and osteoarthritic by the three senior radiologists at the Royal National Orthopaedic Hospital. All but one of the cases with immunofluorescence fell into the latter group.

The results thus support the hypothesis that plasma cells in the synovium in cases of osteoarthritis produce immunoglobulins. The similarity of the immunoglobulins to rheumatoid factor may be accounted for by the widely accepted hypothesis that rheumatoid factor is a response to denatured or damaged protein (the reason for the tissue damage still being uncertain), and that tissue damage in osteoarthritic joints leads to an immune response of similar character: Glynn's hypothesis of enzyme breakdown of tissue in osteoarthritis would fit well with this theory.²

We thank Drs E. J. Holbrow and Peter Johnson for help and guidance, Drs R. O. Murray, D. Stoker and M. Chapman who read the X rays, and Drs Eftekari, Kashani and Costa de Silva who located and presented the X rays.

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reviews

Atomic energy in the US

Michael Flood

The Menace of Atomic Energy. By Ralph Nader and John Abbotts. Pp. 414. (W. W. Norton: New York, 1978.) Hardback \$10.95.

THE most destructive myth of the twentieth century is undoubtedly the myth of the "Peaceful Atom". This is the conclusion of Ralph Nader's latest book, *The Menace of Atomic Energy*. The "Peaceful Atom", according to Nader, was a mistake from the start. The US Government pushed and cajoled the electrical utilities into accepting it by promising that fission technology would be cheap—one executive even suggested that electricity from the atom would be "too cheap to meter". The government subsidised the fledgling industry, protected it from interference and competition and promoted it at home and abroad with an aggressive sales pitch. It "artificially contrived an environment in which utilities were made to fear that hesitation to 'go nuclear' would put them in an uncompetitive position". Nader suggests that today many of these companies will be bitterly regretting that they yielded to government pressure.

The book is co-authored by John Abbotts, a Princeton graduate who served his apprenticeship as a nuclear submariner; his experience supervising nuclear reactor operation must have deeply influenced his views. Mr Abbott's scientific training more than adequately complements Nader's legal mind; his influence is apparent, especially in the clarity of the technical arguments and descriptions. It is no easy matter explaining to a lay audience the intricacies of nuclear reactor operation. (It is a pity that less effort went into the diagrams; freehand sketches (two wrongly labelled) are casually interleaved with direct replications of complicated engineering drawings.)

The book is well written, accurate, and, as we have come to expect from Nader, hardhitting and uncompromising. It is divided into four sections. The longest part, Section II, presents "The Case Against Nuclear Power"; Section I contains a short history of America's nuclear programme together with an overview of the issues. Section III is a profile of the American nuclear industry and Section IV, "What Can a Citizen Do?", a consumer's guide to

action. The pages are liberally spiced with stories of government cover-up and industrial complicity, and embellished with snappy personal case-histories and vivid descriptions of incidents. Those who come fresh to the subject and who are unfamiliar with the history of the nuclear industry will find the book makes disturbing reading—it is, to quote the cover flap, "a chilling book". Seasoned campaigners—and the book is clearly aimed at a wide audience—will find very little new in the book, although the authors have, to their credit, assembled a useful catalogue of the issues and arguments, well referenced and well indexed.

For the general reader, the best section in the book is the second, in which Nader and Abbotts outline the risks of and the technical, economic, social and political arguments against nuclear development. The discussion of the hazards of reactor operation is logically sandwiched between sections on the 'front-end' and 'back-end' of the nuclear fuel cycle. Too frequently, as the authors show, the mishandling of nuclear materials, or ignorance of their biological effects, has led to unnecessary exposure to ionising radiation, described in the book as "the silent violence perpetrated on the workers throughout the nuclear fuel cycle". The inhalation of gases and dusts in the uranium mines has led to "an epidemic of respiratory cancers" among miners. Workers families in some parts of the southern US have also been exposed to drinking water contaminated with radioactivity (from spoil-heap run-off) at levels far above those permitted by law.

The record of radioactive waste management in the US has been appalling; containment failures during storage are on a scale that make the continuing saga of the leaking Windscale silo pale into insignificance. Spills of high-level liquid waste at the Hanford reservation alone have totalled about 500,000 gallons. The site has become extensively contaminated, to a degree where even the jackrabbits have begun spreading the stuff around. As an official explained, investigators recently located one "hot spot" which turned out to be the carcass of a coyote—it wasn't clear how it had died, whether from natural causes or a surfeit of rabbits

Supporting their technical case on nuclear reactor safety, Nader and Abbotts cite the testimony of a number of "whistle blowers", top-rank nuclear engineers and physicists who have defected over the past four years. One defector, Robert Pollard, a senior electrical engineer with the Nuclear Regulatory Commission, resigned his post in January 1976 complaining that the Commission was "blind" to the unresolved reactor safety issue. In his letter of resignation, Pollard stated that: "generic unresolved safety problems are so fundamental to the evaluation of reactor safety that it is not possible to conclude on a technical basis that operation of any nuclear reactor is safe enough to provide reasonable assurance of adequate protection for the public health and safety".

One reason for the concern over safety is that the utilities have been forced to cut corners as a result of increasing economic pressures. According to the authors, the utilities became hooked on nuclear power before they realised its true costs. The gap between estimated and actual costs is already substantial and growing fast (as are government subsidies). Systems reliability is poor and deteriorating, and utilities are facing a crisis of confidence from the money suppliers. And, as if this were not enough for the beleaguered nuclear industry, interne-cine warfare has broken out with Westinghouse, one of the biggest reactor vendors, being sued for breach of contract by a number of utilities and itself suing 29 foreign and US uranium producers, charging them with illegally raising and fixing uranium prices.

The concluding sections of the book present a good profile of the nuclear-electric industry in America together with a detailed analysis of the mechanism by which the various regulatory and promotional bodies interrelate. Two chapters review possible citizen action at a federal level, in Congress and through the courts; a third, the possibility of exerting pressure at state and local levels through initiatives and referenda; and a fourth examines direct challenges to utilities engaged in the construction of nuclear plant.

Despite its virtues, the book suffers from a number of serious flaws. Most conspicuously, the book is unambi-

guously American in context and in its assumptions. It would have benefited greatly from a more international perspective. In places its assessment of theoretical work is uncritical and lacks objectivity. For example, the authors lay great store by the work of Gofman and Tamplin, two scientists formerly employed by the US Atomic Energy Commission, and in particular their estimates that natural background radiation levels in the US already cause some 19,000 cancer and leukemia deaths with as many as 588,000 deaths resulting from heart disease or diabetes as a result of radiation-induced genetic damage. Whatever the merits of the case—and the issues are complex and difficult—the theoretical work of Gofman and Tamplin has been strongly criticised and contested. It would have been helpful to have heard a little more about the precise nature of these objections.

The chapter on "Alternatives to Nuclear" is poor. In their enthusiasm for solar, wind and energy conservation technologies the authors present an uncritical and eclectic assessment of the options with scant regard to economic or potential environmental or social impact. The chapter gives every appearance of a rush job, and this is doubly unfortunate in view of the importance of the issue and the strength of the case that can be made.

Finally, by concentrating their efforts on such questions as reactor safety, waste disposal and the environmental impact of radioactivity, the authors have failed to give due attention to what is undoubtedly the most serious and most immediate global

problem associated with the development of civil nuclear power, nuclear weapons proliferation. Proliferation is the real "menace of atomic energy", as politicians are now beginning to recognise. President Carter was quick to sound the alarm back in April 1977 when he announced that commercial reprocessing in the US would cease forthwith. Since then he has initiated the International Nuclear Fuel Cycle Evaluation to explore possible alternative pathways. Jimmy Carter has had first-hand experience of nuclear power: like Abbots he too served aboard nuclear submarines. He was also one of those drafted in to help clean up a serious nuclear reactor accident at Chalk River in Canada. His experience may well have helped shape his current views that atomic energy should be used "only as a last resort."

"Atoms for Peace" and "Atoms for War" come from the same mould. All attempts to break the link between these Siamese twins by imposing international safeguards on the supply of sensitive technology and materials have proved to be ineffectual. For Nader and Abbots, Carter's April 1977 speech, in which he announced his policy changes, came too late; their book had gone to press.

The Menace of Atomic Energy is a book that is unlikely to ruffle too many feathers on this side of the Atlantic. But in America, where Nader has a strong and vociferous following, it will compound the problems facing that self-professed "multi-million dollar underdog", the American nuclear industry. □

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The next important piece of work of Albert Szent-Györgyi was concerned with the oxidation and reduction of the C₄-dicarboxylic acids—that is, succinic, fumaric and malic acids—which was carried out during the period 1934–1936. He introduced the use of minced pigeon-breast muscle into biochemistry, and showed that these substances mentioned had a catalytic function in biological oxidation. These important observations could only be fully interpreted by H. A. Krebs, who published the main outlines of the tricarboxylic acid cycle in 1937. Krebs in the present volume pays a generous tribute to Szent-Györgyi in an essay which develops the thesis that scientific progress does not occur in a straight line, that false trails are difficult to avoid, and that many errors arise from erroneous but plausible premises.

Two other articles in the section headed "Metabolism" are by F. Lipmann "On Some Unusual Nucleotide Polyphosphates", which contains some unpublished data, and the other by H. M. Kalckar entitled "Ectobiology. Transport of Nutrients, and Chemotaxis". Both these short papers contain what seem to be some new and challenging ideas.

The greatest scientific achievement of Albert Szent-Györgyi was, however, in the biochemistry of muscle. Engelhardt had shown in 1939 that myosin splits ATP, and Szent-Györgyi in the early 1940s, together with Straub, demonstrated that "myosin" is really a mixture of myosin and a new protein to be called actin. He also obtained acto-myosin threads, which contracted in the presence of K⁺, Mg²⁺ and ATP. These and other observations laid the foundations of modern muscle biochemistry. Further developments in this area are discussed in articles by H. E. Huxley, Gergely, Andrew Szent-Györgyi and others. The volume also contains a short but critical and forward-looking review on troponin and its function by Setsuro Ebashi, who has contributed so much to our knowledge of the function of calcium and the sarcoplasmic reticulum in muscle contraction. These articles also indicate how Albert Szent-Györgyi has contributed throughout his life to the enhancement of our knowledge in this field by his stimulating and critical support of other workers. Another paper, by Lorand, deals in an interesting manner with transamidating enzymes which are involved in forming cross-linkages between proteins, a phenomenon particularly important in fibrin formation. Another paper by Isenberg deals with the "inner" histones, their aggregation and interaction with chromatin.

A completely different topic, a novel assay of reticulo-endothelial function, is presented by C. B. Huggins and A. H. Reddi. The book also contains essays of more theoretical physicochemical character, such as the contributions of M.

Tribute to Szent-Györgyi

Search and Discovery: A Tribute to Albert Szent-Györgyi. Edited by B. Kaminer. Pp. 344. (Academic: New York, London and San Francisco, 1977.)

ALBERT SZENT-GYÖRGYI was born in Budapest in 1893; he was Professor of Biochemistry first at Szeged and later at Budapest. After the end of the Second World War he moved to the United States and established an Institute of Muscle Research at Woods Hole. His scientific activities ranged over an unusually wide field, covering vitamin C, intermediary metabolism, the biochemistry of muscle, the application of quantum mechanism, and cancer. His boundless energy and enthusiasm, his remarkable intuitive capacity, the wide range of interests, and his generosity to his students and colleagues, have made him an influential leader in the field of

biochemistry all the world over. It was therefore fitting that his former students and many of his colleagues should hold a Symposium in his honour in Boston, and the papers given on that occasion have now been published.

The first very important piece of work of Szent-Györgyi was the isolation of vitamin C in crystalline form in 1928. He showed that the strongly reducing substance was the antiscorbutic substance for which many scientists had been searching. In the present volume Linus Pauling presents his views (which have been given elsewhere) that a high intake of vitamin C (1–6 g per day) leads to improvement in health. He produces arguments and data in support of his belief that such a high intake of this vitamin has both a curative and protective effect on some bacterial diseases, coronary thrombosis and cancer. One must respect the views of Pauling, who is a scientist of great eminence, but it must also be stated that he has not yet persuaded the bulk of nutritional scientists that these opinions are indeed correct.

Kasha, Alberte Pullman and Bernard Pullman. There are also various articles on topics such as gene expression and cancer. Thus, Seymour S. Cohen contributes a challenging and stimulating essay on the strategy of chemotherapy of virus diseases and cancer.

I have not mentioned several other papers of interest in this volume, but I must refer to an essay by Albert Szent-Györgyi himself on "Electronic Biology and Cancer". This is a *tour-de-force*, in which the author, on the basis of simple but somewhat primitive physical concepts, engages in a far-reaching speculation on the nature of cancer, and relates it to the action of glyoxalase. Both the

theory and experimental support are at present sketchy, but the essay may possibly stimulate others to further explore the role of free radicals in biology, and particularly cancer research, with adequate methods.

The book is well produced, contains many articles of great scientific interest, will give a lot of stimulus and enjoyment, and will occasionally produce some irritation in the critical reader.

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High-altitude peoples

Biology of High-Altitude Peoples. (International Biological Programme, Vol. 14.) Edited by P. T. Baker. Pp. 357. (Cambridge University Press: Cambridge and London, 1978.) £19.50.

A SUBSTANTIAL number of people, though no-one knows exactly how many, live at high altitudes (often defined as above 2,500 m), mainly in South America but also in eastern Africa and Central Asia, including the Himalayas and associated mountain chains. Many are living at or near the limits of human survival. Because of this and because there is little technological capacity to ameliorate one of the dominant environmental factors—low levels of atmospheric oxygen—they afford one of the best groups for analysing the biological components of human adaptation. It was for this reason that they were singled out for special consideration in the Human Adaptability section of the International Biological Programme. This book summarises and synthesises the results of those studies. Most of the work was undertaken by North and South American workers in the Andes, but other investigations were launched in Ethiopia, Nepal and Bhutan, and in the Tien Shan and Pamirs of the Soviet Union.

The approach in this book, however, is, in the main, to present the results by topics such as genetics, growth, work capacity, haematology, nutrition and cold. This has the advantage of providing the reader with a broad view of the processes involved in high altitude adaptation and the components of fitness, but it also emphasises the surprising differences which are to be found between peoples of different geographical region but similar altitude zone. There is certainly no single blueprint for altitude survival and it would seem that many local factors including

ancestry and history are involved in determining the biology of mountain peoples.

Individual physiological acclimatisation to reduced availability of oxygen is a well demonstrated and functionally important aspect of high altitude living, but there is also much suggestive evidence that indigenous highlanders are better adapted to their environment than fully acclimatised lowlanders. Regrettably no specific genetic system has yet been identified which can account for this, and some of the phenomena may be due to developmental processes rather than genetic ones. Nevertheless, there would seem to be heritable components to some of the morphological, biochemical and haematological characters which distinguish at least Andean highlanders, and it seems likely that natural selection has played an important role in producing their adaptations.

All the contributions in this book are good and it may be invidious to single out any for special mention. But I did find the ones on fertility and early growth by E. J. Clegg and on childhood growth by A. R. Frisancho particularly useful in understanding how high altitude phenotypes develop. The IBP provided a great impetus to comparative growth studies. The book also provides one of the few accessible accounts of physiological work that Soviet scientists have been undertaking in Tien Shan, and the final chapter by the Editor on adaptive fitness is a thoughtful and penetrating discussion of how demographic data can be used to gain holistic insights of the nature of population adaptation.

The book, which is attractively produced, can be most strongly recommended. It is a fitting tribute to the IBP and will for many a year be the standard source on the human biology of high-altitude peoples.

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Electron spin resonance from the ground up

Theoretical Foundations of Electron Spin Resonance. By John Harriman. Pp. 397. (Academic: New York and London, 1978.) \$39; £25.35.

THIS is electron spin resonance from the ground up, which, as far as we need be concerned, is the Dirac equation. Dr Harriman has written a text which is unashamedly theoretical, and in which the thrust of the development is to start at the beginning, to go on to the formulation of the spin-hamiltonian, and then to end with the interpretation of the parameters in the spin-hamiltonian in terms of the electronic structures of the species under study. The species he has in mind are inorganic and organic free radicals in which orbital effects are largely quenched; in other words, this is more a book for theoretical chemists than for physicists—hence the word *Spin* in the title. The text is confined to isolated, stationary species; relaxation processes are not considered.

The treatment is most welcome. There is much to be said for a complete, thorough, detailed, sustained and largely uncompromising survey of this field, and the place it takes in the literature has not previously been occupied by any of the other books on electron spin resonance. Even workers in the field who have no pretensions as theoreticians ought to spend some time reading the helpful summaries at the end of each section.

One of the attractions of spin resonance for the theoretician is that the central object of study, the electron spin, is well defined and has only two energy states; and even in the next order of approximation (taking account of the effects due to nuclei) the number of levels is small. The energy levels can therefore be treated in great detail, but to do so effectively the whole bank of operator techniques has to be drawn on. In other words, the problems that not only can be solved but, on account of the sensitivity of the technique, have to be solved, can be tackled fairly easily by sufficiently sophisticated methods.

Electron spin resonance provides good, tractable problems for the theoretician, and they are problems that the experimentalist needs answered. All this comes out very clearly from Dr Harriman's extended, thorough treatment of the subject.

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Essence of biophysics

Molecular Biophysics. By M. V. Volkenstein. Pp.621. (Academic: New York and London, 1977.) \$36.50; £25.90.

PROFESSOR Volkenstein's many friends and admirers will be delighted that his latest book, published in its original Russian version some two years ago, is now accessible to a wider scientific public. Although intended as a more or less introductory text, it displays the same lucid and seemingly effortless scholarship which characterised his earlier and classic treatises *Molecular Optics*, *Vibrations of Molecules* and *Congrational Statistics of Polymer Chains* (which is unaccountably the only other one presently available in English translation).

Volkenstein stands in the great tradition of Russian theoretical physics, and early in his career came under the influence of such men as Landau, Tamm, and particularly Mandelstam, in whose laboratory he made his reputation with contributions of classic stature on the theory of optical activity and on Raman spectroscopy. In the early 1950s he was drawn, along with many of his most brilliant contemporaries, into polymer science, and with Tsvetkov, Frenkel, Frisman and Bresler, created in Leningrad a major centre of research in this area. There Volkenstein attracted a group of exceptional younger workers, including Ptitsyn, Birshtein, Gotlieb and Eisner, and inevitably they turned their attention increasingly to the rapidly burgeoning field of biopolymers. As one of many visiting workers from all over the world, I had the privilege of witnessing the excitement of this new venture and experiencing the intensity of the intellectual ferment which Volkenstein is unfailingly able to generate.

Characteristically Volkenstein has progressed from his initial preoccupation with the structure and conformational transitions of biopolymers into central questions of biological function. In the introduction to this book he gives a definition of a biophysicist, or perhaps a *credo*: "A biophysicist is a physicist possessing broad biological erudition, who is also able to formulate and solve physical problems. Biological erudition, implies more than the knowledge of the special fields of biology bearing directly on the subject at hand, say molecular biology or physiology. One who is alien to living nature, who is not familiar with zoology and botany, does not understand biology". Erudition indeed is a quality that Volkenstein possesses in abundance.

His emphasis is essentially scholarly, rather than pragmatic, and in this he follows the traditional precepts of the Russian school of physics. His book thus concerns itself more with the creation of a satisfying intellectual framework than with the detailed description of phenomena. It concentrates also on the topics that can usefully be treated with some critical rigour, which makes it less important that the references stop at 1975.

The book deals with the basis of the physical methods used to study proteins and nucleic acids in both structural and dynamic terms. It is particularly strong on cooperative phenomena, but the broader physical principles of enzymology and biosynthesis are not neglected. Theoretical arguments are developed in relatively leisurely fashion, but the more tedious mathematical manipulating are omitted. The result is a very readable book, which carries the reader along very compellingly. The translation (by an anonymous hand, presumably that of the author himself) is smooth, and, as

far I am capable of judging, in excellent style.

To my knowledge, there is no rival textbook in the field. Volkenstein's coverage is more mathematical and rigorous than, for example, van Holde's *Physical Biochemistry*, and it assumes a grounding in physical science at the degree level. It therefore fills a real need, and cannot help but appeal particularly to physicists and physical chemists entering biological research. The book is well produced and reasonably priced by today's rather alarming Western standards. I anticipate that many graduate students and research fellows will have good reason to be grateful to both the author and his publishers for many years to come.

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Scanning electron microscopy

Introduction to Biological Scanning Electron Microscopy. By M. A. Hayat. Pp.323 (University Park Press: Baltimore, Maryland, London and Tokyo, 1978.) £9.25.

IN the first sentence of this book the author acknowledges that it is a most demanding task to write a relatively short volume aimed at both beginning and experienced workers. Hayat is unfortunately hoist by his own petard, for this book would be confusing for the beginner and is infuriating for the experienced microscopist. The volume is split into two parts: the first deals briefly with the instrumentation of the scanning microscope, the second and longer part with specimen preparation.

The first section on instrumentation, though covering most of the main points, does so in such a spartan manner that a beginner would find it difficult to know how to operate the instrument to obtain maximum information from his specimen. The author clearly considers biologists are innumerate, for this whole section is devoid of any equations, which surely must be the most concise way of explaining electron optics.

The section on specimen preparation is no more than a compilation of other people's work and with the exception of the section on fixation there is little to persuade the reader that the author has had first-hand experience with the techniques he describes. Although numerous recipes are given for the various manoeuvres of specimen preparation, the author

is insufficiently incisive in separating fact from conjecture and fails to critically assess the relative merits and faults of the different techniques. The beginner would be left floundering trying to decide on the best course of action, and the experienced microscopist would find little recourse to refer to this section.

The text is sadly unbalanced. Most of the methodology is directed towards mammalian tissue, with only lip service given to invertebrates, plants and micro-organisms. Stereo techniques, which are at the heart of quantitative microscopy, are dismissed in half a page, whereas over 20 pages are devoted to cataloguing cell surface labelling methods. Eight pages of pictures are devoted to illustrating the various containers which may be used during critical point drying, but no pictures illustrate the section on the artefacts which can arise during specimen processing. The scanning microscope invariably provides visually satisfying images, and it is sad that the author has limited himself to 20 rather poor scanning micrographs to illustrate over 300 pages of text.

Regrettably, this is a far from satisfactory book, and cannot be considered as a *vade-mecum* for biological scanning microscopists. Experienced microscopists will quickly realise that it lacks any authority and the beginner will find that it gives neither the rationale behind applying a particular course of action nor a concise statement of how best to carry it out.

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obituary

C. H. Best

THE cliché 'end of an era' seems unavoidable in speaking of the death on 31 March 1978, of Charles Herbert Best, F.R.S. In 1921, a 22-year-old medical student, he was the junior member of the Banting and Best pair that discovered insulin.

The events of the episode have often been described. Frederick Banting, unsuccessful in medical practice, sold his office furniture, books and instruments and came to the University of Toronto. His objective was to obtain the blood-sugar-lowering principle that was thought to be present in islet cells of the pancreas. He asked the physiology professor, J. J. R. Macleod, for the use of a laboratory for eight weeks, for 10 dogs, and for an assistant who knew chemistry and physiology. The assistant was a volunteer, Charles Best. The budget was \$100. Macleod went to Scotland for the summer. Banting and Best stayed in Toronto and bought stray mongrel dogs for a dollar apiece. They ligated pancreatic ducts of some of the dogs to destroy tissue that produced the proteases that would hydrolyse the hypothetical substance when the glands were later excised and macerated. After several weeks and many failures, they prepared an extract that lowered the blood sugar of de-pancreatized (and therefore, diabetic) dogs.

The dreadful and helpless ordeal of early death in human diabetes could now pass into history, as the implementation and improvement of the finding swiftly followed, aided, of course, by other scientists. A major advance was J. B. Collip's procedure of extracting beef pancreas with alcoholic HCl. In 1923, the Nobel Prize in Medicine was awarded to Banting and Macleod, as head of the Department. Banting, noting that his colleague was overlooked, divided his share of the prize with Best, following which Macleod divided his share with Collip.

The rest of the story of insulin unfolded through the years, as a host of talented and well-funded scientists worked diligently and brilliantly to discover the functions and properties of insulin, including its amino acid sequence, crystallographic structure, and formation from pre-proinsulin. Insulin was the first great affirmation that biochemistry could rival bacteriology in preventing a disease that here-

Best (left) and Banting in August 1921 with one of the first de-pancreatized dogs to have its life prolonged by insulin. The photograph, taken on the roof of the University of Toronto Medical Building, is reproduced by courtesy of the C. H. Best Institute, University of Toronto.



before had killed millions of defenceless victims. The effect of the discovery was incalculably great, as a stimulus to public support of research, especially in Canada, in attracting students to laboratory research careers, and in removing feelings of helplessness.

Many great life-saving discoveries came after insulin. In the years following 1945, DDT saved more lives and prevented more illnesses than any single chemical in history, and then fell victim to denigration and obloquy. Its benefits were exceeded only by the antibiotics which are now also under attack for 'spreading resistance.' Discovery and synthesis of the vitamins halted many deficiency diseases, but today vitamins are sold as placebos. So far, insulin has escaped such downgrading although there are mutterings, among non-diabetics, that by keeping people alive, it sets aside the harsh but pre-ordained role of natural selection. The best answer to this is to be found in the achievements of eminent diabetics since the 1920s their lives

preserved by insulin.

The wide acclaim that came to Best is well illustrated by an unparalleled ceremony half a century after 1921: a commemorative and thanksgiving service for diabetics held in St Paul's Cathedral, where Dr Best read the Lesson from the scriptures.

Next year, in a San Francisco hotel room, he told me, his former student, about this. He said that the Lesson at that service was from chapter 17 of 1 Kings, 17-24. It recounts how the prophet Elijah revived the unconscious child of a widow, after she had upbraided him for bringing disaster upon her house and sickness to her son. Elijah then restored the son to life and said, 'See, thy son liveth' and the woman said to Elijah 'Now I know by this that thou art a man of God.' Symbolically, and in an updated setting, the San Francisco hotel employee who carried Best's suitcases refused a tip, because, he said, 'My mother has diabetes.'

Best served in World Wars I and II. He was chairman of the Department

of Physiology at Toronto from 1929 until his retirement in 1965. He directed the Best Institute, and the Banting and Best Department of Medical Research, following the death of Banting in 1942. He maintained his interest in insulin and carbohydrate metabolism throughout his career. He also published research on histamine and heparin, and on choline and fatty livers. He was a Canadian, born in Maine, and was much sought as a lecturer on both sides of the Atlantic. He was a man of true humility, dignified, yet buoyant and outgoing; reserved, yet friendly and informal with all who met him. He was filled with a youthful enthusiasm and was always devoted to research. He married Margaret Mahon in 1924 and they had two sons, one of whom, Charles Alexander, predeceased his father by a few days.

A climber once said: 'I have no doubt that others will follow our track to the summit . . . but never again will any one feel the inspiration, the excitement and the glory of success that we three experienced when the first ascent was made.' Such a moment must have come to Banting and Best in July 30, 1921, when the color reaction showed a steady drop in a dog's blood glucose during the hours following the first injection of insulin.

Thomas H. Jukes

G. J. Hoytink

PHYSICAL chemists everywhere have been saddened to learn of the sudden death, on 11 April 1978, of Gerrit Jan Hoytink, Professor of Physical Chemistry in the University of Sheffield.

Jan Hoytink was born in Alkmaar, North Holland, on 14 January, 1925. He studied at the Free University in Amsterdam and after presenting his Ph.D. thesis in 1952 was appointed Lecturer in Physical Chemistry in 1953. In 1957 he became the first Professor of Physical Chemistry at the Free University and in 1960 moved to the Chair of Physical Chemistry at the University of Amsterdam. He remained there for six years before moving to Sheffield.

The theme of all his work was to account for the properties of conjugated molecules, usually aromatic hydrocarbons, using molecular orbital theory. This began immediately with his Ph.D. research, in which he investigated resonance energies by interpreting heats of combustion and the energies of the lowest singlet-singlet transition for several types of conjugated system. His first independent work, which was to quickly establish his reputation, was to measure the reduction potentials of aromatic hydro-

carbons and interpret them using semi-empirical molecular orbital theory. It was at about this time that it was first reported that the coloured compounds formed on exposure of hydrocarbon solutions to alkali metals were paramagnetic and he quickly realised that the species formed in the two types of reaction were identical.

He went on to appreciate the significance of the pairing theorem for spectroscopic properties of radical-anions and cations, to interpret the optical spectra of radical-anions using molecular orbital theory with configuration interaction, and to illustrate how configuration interaction gave a good account of the appearance of negative spin populations in certain radicals and radical-ions. He thus played a seminal role in opening up the field of radical ions, systems which for the last ten years have rated their own biennial Gordon Conference. He became interested in triplet states, in particular ground-state triplets expected for the dinegative ions of hydrocarbons of high symmetry, in radiationless transitions, and in radiative transitions of mononegative ions. Latterly he used nano-second and ultimately pico-second laser flash photolysis to probe still further the excited states of hydrocarbon anions.

He had a high-minded view of his own work: he liked things to be done properly and would not spend time on things he regarded as trivial just to create an effect. Similarly with the teaching of physical chemistry, he was most unhappy with any suggestion that one might try to (over) simplify things in order to give the students an easier ride. This commitment to intellectual rigour has left its mark in at least two ways. First, in the high standards of assessment which he set as an early Chairman of the Dutch Chemical Foundation, and which prevail to this day. Second, in the standard he set for *Chemical Physics Letters*, the journal which he encouraged North-Holland to publish, and whose editor he was from its foundation in 1967. In this he had little sympathy for offerings where he detected a hint of entrepreneurship but was disposed to look with a kindly eye on anything he regarded as an honest effort, particularly, perhaps, if the authors were felt to be in a situation where it was difficult to do any research at all. He used to say that he thought the important thing was that a paper would be stimulating. This was presumably not the whole of his editorial philosophy, but in any event he delivered, nursed, and developed a most successful and highly regarded journal.

Jan Hoytink did not always live easily with the world or with himself, and his life was turbulent. On the one

hand he needed to be true to himself and tell things as he saw them, on the other, his nature was not such as to insulate him from the natural shocks that flesh is heir to. Through all, he was a good man, who illumined science and life for his many friends.

N. M. Atherton

A. D. Thackeray

ANDREW DAVID THACKERAY, former Director of the Radcliffe Observatory Pretoria and one of the most distinguished British astrophysicists, was killed in an accident on 21 February, 1978.

David Thackeray was born in 1910 and was educated at Eton and Kings College Cambridge. His early research work at Cambridge and at the Mount Wilson Observatory California was on various topics in solar and stellar spectroscopy. He made an early and outstanding contribution to astrophysics by resolving a long-standing problem relating to Mira-type variables. Some emission lines found in the spectra of these stars show quite unusual relative intensities. It occurred to him that this was the effect of resonance excitation and he worked out a detailed explanation which still holds. He joined the staff of the Solar Physics Observatory Cambridge in 1937. The end of World War II found him serving with a Quaker Unit in Italy.

In 1948 he went to South Africa as Chief Assistant at the Radcliffe Observatory Pretoria. The 1.9 m reflector, then the largest in the Southern Hemisphere, was just coming into action and he was to devote the rest of his life to the full exploitation of this telescope. He succeeded Dr H. Knox-Shaw as Director in 1950 and remained in that post till the Pretoria site was closed in 1974. The 1.9 m reflector was acquired at that stage by the South African Astronomical Observatory for its main Observatory at Sutherland Cape Province and David Thackeray became Radcliffe Professor of Astronomy at the University of Cape Town where he remained active in astronomical research till his death. His many publications cover almost all aspects of astronomy and only a few can be mentioned.

He discovered (with A. J. Wesselink) that the Magellanic Clouds (the two nearest galaxies) were twice as distant as previously thought. He announced this at a Rome meeting in 1952. At this meeting the American astronomer, Baade, announced a similar result for the Andromeda Galaxy but on less certain grounds. These results doubled the accepted age of the Universe and

resolved the paradox that the current age was smaller than the age of the Earth. His work on the Magellanic Clouds also showed that they were a mixture of both old and young stars contrary to earlier suppositions. In contrast his work on the Sculptor Galaxy was largely responsible for establishing that such spheroidal galaxies consisted entirely of old stars. With his collaborators he made extensive spectroscopic studies of stars in the Magellanic Clouds. This established an upper limit to stellar brightness at about a million times the solar luminosity. Stars at this limit showed evidence of instability. Again with his collaborators, extensive radial velocity measurements were made in Pretoria of stars in the Southern Milky Way. This led to new determinations of the constants of galactic rotation and an estimate of the distance to the galactic centre.

Although active in many fields David Thackeray regarded himself primarily as a stellar spectroscopist and his knowledge of this field was encyclopaedic. He was particularly interested in objects with unusual spectra and the significance of this work is only slowly being realised. Thus his investigations over many years of the eclipsing symbiotic star AR Pav is a major clue to the understanding of symbiotic stars. His main legacy in this field is his studies extending over nearly 30 years of the enigmatic exploding object Eta Carinae and the slow nova RR Telescopii. In these objects he identified forbidden emission lines not yet produced in the laboratory and his detailed studies must form the basis of any theory of these puzzling objects. His discovery of strong polarisation in the halo around Eta Carinae is of particular importance.

David Thackeray approached observations with an open mind and complete integrity; refusing to draw any conclusions which were not completely justified. He was remarkably observant and rarely failed to notice an interesting feature in a spectrum even on a brief examination (often in the darkroom when the plate was still washing). In 1949 he noted visually with the 1.9 m telescope variation in brightness of VV Pup on a time scale of seconds. This seemed hardly credible at the time but 20 years later the result was fully confirmed using high speed photometers and is an important property of cataclysmic variables.

David was an enthusiastic observer of all natural phenomena and published a number of notes on them. The last of these on 'Dust-Devils and Spray-Devils' was published in October 1977. It was, in fact, a violent dust devil that overturned the vehicle in which he was a

passenger on February 21. He had completed a successful week's observing with the 1.9 m telescope at Sutherland and was returning to Cape Town. He was killed instantly.

He more than any other single person opened up the Southern Hemisphere to modern astrophysics. His work was done quietly and without publicity but it has established his name at the very front of international astronomy.

Astronomers throughout the world will remember David's kindness, courtesy and humour. Many were privileged to enjoy the hospitality of David and Mary and their children in a home where astronomy blended naturally with music. He particularly welcomed and helped the many young British astronomers who came to work in Pretoria and in this way he made an important contribution to the development of astronomy in the United Kingdom.

Long a Fellow of the Royal Astronomical Society (London) he was elected an Associate a few days before his death; the first British subject to be accorded this honour. The news was waiting his return to Cape Town.

M. W. Feast

Victor Eyles

DR VICTOR EYLES, F.R.S.E., who died on 8 March 1978 at the age of 82 was the leading British historian of geology and one of the world's most eminent workers in this field.

He was a native of Bristol and after service during the first world war, first with the Royal Engineers and later as Observer with the Kite Balloons of the Royal Flying Corps, he graduated in 1920 in petrology at Bristol University. He had a long and distinguished career with the Geological Survey of Great Britain (1920-1955). In the years before the war he worked in Scotland and Northumberland making notable contributions to our knowledge of the Carboniferous sedimentary and igneous rocks of Ayrshire and Renfrewshire and the ancient crystalline (Moine) schists of the West Highlands.

In 1940 he was selected to report to the Non Ferrous Ores Committee of the Ministry of Supply on the bauxite deposits associated with Tertiary lavas of Antrim, Northern Ireland, and was later commissioned by the Ministry of Aircraft Production to carry out an extensive programme of exploration by boring. This proved 750,000 tons of bauxite, which, though rich in iron, was of commercial grade. By 1944 about 250,000 tons of the bauxite had been mined, and this produced 35,000

tons of aluminium for British aircraft, freeing valuable shipping space for the import of other essential raw materials. After the war Eyles was based in the London Office of the Geological Survey, in charge of the field units working in the Midland and South Wales Coalfields.

Dr Eyles' eminence as a historian of geology springs from a number of deeply researched bio-bibliographical articles on pioneer geologists and their work. Outstanding examples are the paper on Hutton's original statement of the *Theory of the Earth* and his detailed account of the Swiss geologist, L. A. Necker. Eyles was instrumental in securing the eventual publication of Necker's map of Scotland, which had been completed in manuscript in 1808. Other early geologists whose work has been described by Eyles are J. MacCulloch, Robert Jameson, Sir James Hall and A. G. Werner. Together with his wife, Joan, he wrote a most valuable account of William Smith's great geological map of England and Wales. He also contributed some excellent general articles on the history of science, such as his account of the history of scientific activity in the Bristol region, presented at the British Association's Bristol meeting in 1955. More recently he outlined suggestions for further research in the history of geology in an article published in *History of Science* in 1966, and a summary of his detailed knowledge of the state of eighteenth century geology was set forth in the New Hampshire Symposium published in 1969.

Eyles' interest in the history of his science was greatly stimulated by the fine collection of early geological maps and texts which he and his wife built up over the years. Between 1951 and 1956 he gave courses on the history of geology at University College London, and he lectured at several universities in the USA. He was the United Kingdom representative on the International Committee on the History of Geological Sciences from 1967 to 1972 and was an honorary member of the Society for the Bibliography of Natural History.

Victor Eyles was a man of very few words, every one of which was well chosen. He was one of the most courteous and considerate of men with a wonderfully dry sense of humour, and was prepared to spend almost endless time in providing guidance to others in his chosen field of study. He was definitely a geologist turned historian of geology and his knowledge of both these aspects has made him the doyen of historians of geology in the British Isles.

N. E. Butcher
W. Mykura

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